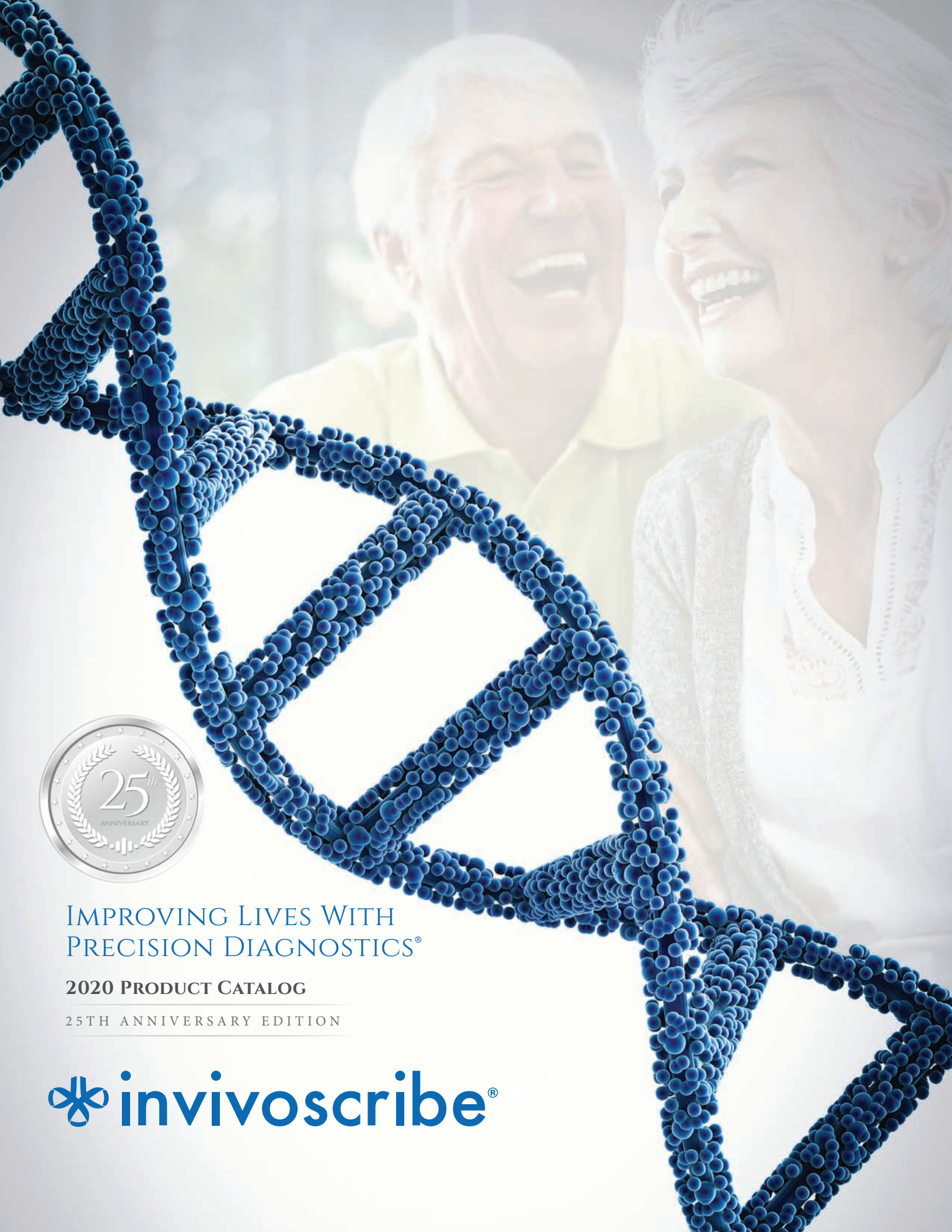
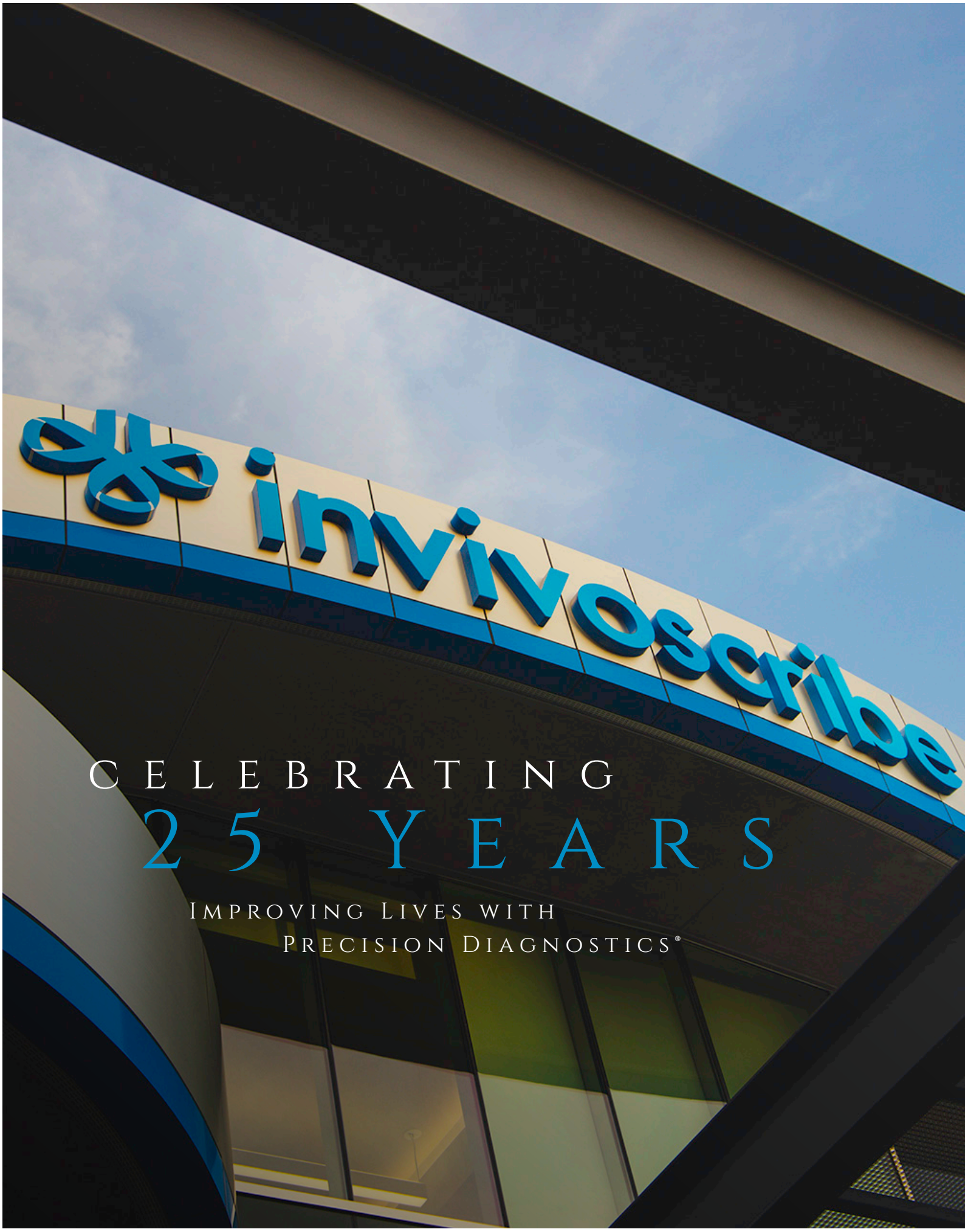


# Improving Lives With Precision Diagnostics®

## 2020 Product Catalog

25th Anniversary Edition

invivoscribe®




# CELEBRATING 25 YEARS

IMPROVING LIVES WITH PRECISION DIAGNOSTICS®

# Dear Colleagues:

I am pleased to introduce Invivoscribe's 2020 Product Catalog. I'll take this opportunity to share some of last year's accomplishments.

Total year over year revenue grew over 30%, supported by new tests, menu items, adoption of our suite of LymphoTrack® assays that include bioinformatics software, and our LeukoStrat® *FLT3* CDx Mutation Assay.

Invivoscribe (IVS) received several regulatory approvals for the internationally-standardized *FLT3* signal ratio test, including approval of the CDx claim for quizartinib in Japan. We submitted a CDx distributable kit package to the US FDA so we anticipate we will be able to sell a *FLT3* CDx kit in the US later this year. We also finalized several new CDx partnership projects: one for a new CDx assay on the 7500 Fast Dx instrument, and one with a previous partner. We will announce these deals early in 2020.

IVS participated in over 30 conferences and tradeshows worldwide, presenting 8 posters and publishing 2 scientific papers with participation from our R&D, LabPMM and bioinformatics groups. A number of scientific papers and posters were also published or presented by customers using our products.

IVS launched MRD controls for both B- and T-cell assays with our comprehensive suite of LymphoTrack Clonality Assays, including LymphoTrack® software for Illumina® NGS platforms. These assays identify and track clonal rearrangements within the antigen receptor loci (*IGH, IGK, TRG, TRB*). The 24 ID format (48 indices for the *IGH* FR1 assay) allows customers to significantly reduce costs, as users can run any combination of the 7 assays (up to 178 samples with 14 controls) simultaneously on one chip, as the accompanying bioinformatics software automatically sorts and interprets the data for each targeted region and sequence ID. These RUO assays are being used with accompanying minimal residual disease (MRD) bioinformatics software in international studies of a wide range of hematologic malignancies, including multiple myeloma and acute lymphoblastic leukemia. The assays will be submitted for review via the US FDA's 510(k) process and with the appropriate regulatory authorities worldwide.

We also finished development of a *TRG* MiSeqDx 510(k) assay; released LymphoTrack® Dx assays for ThermoFisher NGS platforms, and started working under an exclusive agreement with the BIOMED-2 /EuroClonality Consortium in Europe to develop and commercialize their NGS-based assays.

In addition, we made substantial progress building out a new wet lab in Shanghai to add further capabilities for clinical programs to support our pharma partners in China, expanded operations and capabilities at our clinical labs in the US, Japan, the EU, and established an entirely new line of business: setting up a flow cytometry lab so we can soon offer both flow- and NGS-based MRD testing intramurally on exactly the same primary specimens.

We developed a number of engineered cell lines using CRISPR technology, a highly sensitive assay on a digital droplet PCR platform, and filed a number of patents. Still further, we launched a wholly-owned drug development company, Invivoscribe Therapeutics, that has already started testing a small molecule that targets myeloid malignancies.

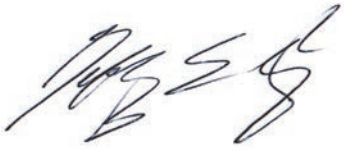
In 2019 our entire range of tests, including MyAML®, and MyMRD® gene panels with our MyInformatics® bioinformatics pipeline, received increased traction and use by more than three dozen pharmaceutical partners. These tests are available as RUO and/or CLIA/CAP-listed tests through our LabPMM clinical laboratories in the USA, Europe, and Asia.

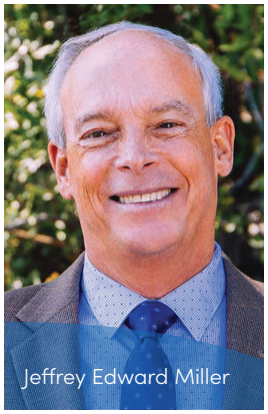
Finally, I want to acknowledge that our continued growth and progress over these past 25 years would not be possible without our customer's feedback and support. We look forward to continued interaction with the research and clinical communities so we can offer you the best products for decades to come and we wish you, your colleagues, and your families a joyful, productive, and successful 2020.

*Sincerely Yours,*

**Jeffrey Edward Miller,** Ph.D.

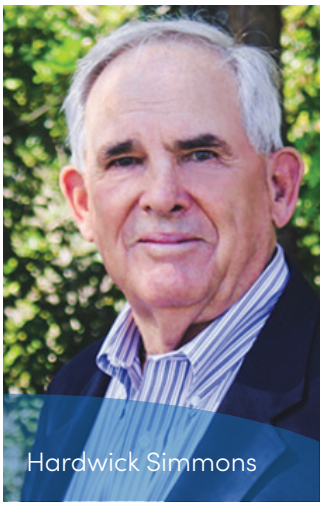
*Founder, Chief Scientific Officer,*
*Chief Executive Officer & Chairman*





Jeffrey Edward Miller

# Invivoscribe
# Board of Directors


Hardwick Simmons

## Hardwick Simmons, MBA

Hardwick 'Wick' Simmons retired as the Chairman and CEO of The Nasdaq Stock Market, Inc. in May of 2003. Prior to Nasdaq, he served as President and CEO of Prudential Securities Inc., a major investment management and securities brokerage firm. Simmons is a former chairman of the Securities Industry Association, a former director of the Chicago Board Options Exchange and a former president of the New York Bond Club. He is currently a director of Lionsgate Entertainment Corp., president of Stonetex Oil Corp., and a trustee of Woods Hole Oceanographic Institution. He is a graduate of Harvard College and Harvard Business School and served in the U.S. Marine Corps Reserve from 1960 until 1966.


Mitchell Kronenberg

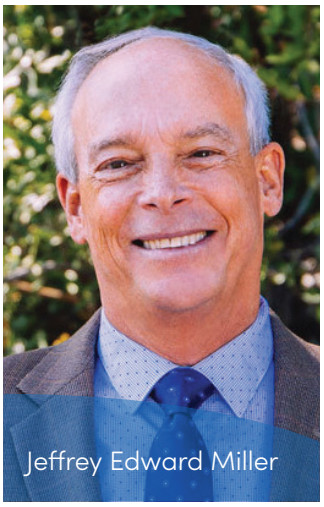
## Mitchell Kronenberg, Ph.D.

Mitchell Kronenberg received a B.A. from Columbia University, a Ph.D. from the California Institute of Technology, and served on the faculty of the UCLA School of Medicine from 1986-1997. He joined the La Jolla Institute for Immunology in 1997, and has been the President there since 2003. The Institute has grown in accomplishment and reputation under his leadership. Dr. Kronenberg's research interests include natural killer T cells, other innate lymphocytes such as MAIT cells and ILC, regulation of mucosal immunology and the microbiome and pathogenesis of inflammatory bowel disease. He has co-authored more than 350 publications, and is a fellow of the American Association for the Advancement of Science (AAAS), a Distinguished Fellow of the American Association of Immunologists, recipient of an NIH MERIT award and is an Institute for Scientific Information (ISI) Highly Cited Scientist. In 2016, he was named the most admired CEO (large nonprofit organization category) by the San Diego Business Journal. He is an advisor to a number of organizations including service as a member of the Board of Scientific Counselors for Basic Science, National Cancer Institute and he has been involved with Invivoscribe since its founding.


Stephen Wilson

## Stephen Wilson, Ph.D.

Dr. Wilson is a biomedical research executive with more than two decades of experience in basic and translational immunology. He received his B.S.A. and Ph.D. from the University of Arizona, and serves as the Executive Vice President and Chief Operating Officer of the La Jolla Institute for Immunology.


Jeffrey Edward Miller

## Jeffrey Edward Miller, Ph.D.

Dr. Miller is a scientist, inventor, and entrepreneur focused on Improving Lives with Precision Diagnostics® – coupling drug trials and therapeutic treatment regimens with optimized clinically-actionable diagnostic methods in order to select the correct patients and then monitor and track their response throughout the course of their disease. He received his undergraduate degree in Biochemistry from UCLA and a combined Ph.D. in Biochemistry & Molecular Biology from UCSB. Prior to starting Invivoscribe, Dr. Miller had more than twenty years of combined experience in protein biochemistry, cellular and molecular immunology, cardiac physiology, virology, and molecular biology, experience he had developed working in laboratories at the Medical School, Department of Chemistry, Molecular Biology Institute at UCLA, while earning his Ph.D. at UCSB, and as a postdoctoral scientist at Applied Molecular Evolution. He also spent several years at Quest Diagnostics Nichols Institute, setting up the molecular oncology laboratory and developing and launching PCR-based molecular assays for infectious disease and hematopathology.


James Isaacs, Jr.

## James Isaacs, Jr., JD.

James B. Isaacs, Jr. has practiced law since 1983. He currently serves as Licensing and Contracts Counsel at Invivoscribe. Jim attended Stanford University and Yale Law School, then began his career at the Los Angeles law firm of O'Melveny & Myers. As a trial lawyer and later in-house counsel with a focus on intellectual property disputes, Jim has successfully defended a myriad of businesses and individuals; in plaintiffs' actions he has obtained and collected multi-million dollar judgments in the United States and abroad. As a businessman and co-founder of Invivoscribe, Jim has been active in the legal and commercial affairs of the company since 1995.


Gary Clouse

## Gary Clouse, JD.

Gary Clouse has practiced as a litigator and business attorney in Southern California for more than three decades. He currently serves as Corporate Secretary and Legal Counsel for Special Projects at Invivoscribe. Clouse is a graduate of Indiana State University and Northwestern University School of Law. Following law school, he clerked for the federal Seventh Circuit Court of Appeals in Chicago. He began his legal practice at the law firm O'Melveny & Myers in Los Angeles. Clouse is one of the founders of invivoscribe.

## Improving the quality of healthcare worldwide.

Committed to providing high quality, reliable, cutting-edge tools for molecular diagnostic and personalized molecular medicine.

# Executive Leadership Team


Meghna Bhatnagar

## **Meghna Bhatnagar, MBA,** Chief Financial Officer

Meghna Bhatnagar joined Invivoscribe in 2010 as Chief Financial Officer. In this role she is responsible for leading the Invivoscribe global finance organization, along with human resources and information technology. Since her arrival, Ms. Bhatnagar has played an integral role in directing all aspects of company strategy, planning and operations.

Ms. Bhatnagar has over 20 years of experience building and leading finance and IT teams in global companies. Prior to joining IVS, she served as COO of Radiant Technologies, a technology company focused on providing business management solutions to small and medium sized companies where she was responsible for leadership and development of an entire project delivery team with full P&L responsibility. She played a key role in guiding overall strategy of the company and at the same time provided leadership for operational improvements.


Dr. Bradley Patay

## **Dr. Bradley Patay, M.D.,** Chief Medical Officer

Dr. Patay is dedicated to improving health by integrating genomic knowledge into medical care. He has authored numerous articles in this field, presented at multiple conferences and has been featured in Bloomberg Business Week. He has been head of the internal medicine section at Scripps Torrey Pines, worked as an Assistant Professor at STSI, and has been a founding member of the Board, and Vice President of the College of Genomic Medicine, which was established in 2010 to educate physicians and other health care professionals about genomic medicine.

His diverse clinical experience prior to joining Scripps Clinic in 2005 includes four years as an internist and pediatrician at Neighborhood Healthcare, a private, nonprofit community healthcare practice, and at Palomar Hospital. At these institutions, Brad cared for a wide range of patients, from neonates to the elderly, in both intensive care and the general wards. Through his service on several committees, he helped improve health care institutions' systems.


Paul McMullin

## **Paul McMullin,** Head of Global Sales & Marketing

Paul McMullin joined Invivoscribe in 2018 as the Head of Global Sales & Marketing. In this role, he leads Invivoscribe''s worldwide Sales and Marketing efforts with focus on both Diagnostic and Companion Diagnostics (CDx) Products. These Products are sold in over 100 Countries via a Direct Sales Organization as well as Exclusive Distributors.

Mr. McMullin has over 35 years of experience working in the Medical Diagnostics Business. Most of that time has been in the molecular and biotechnology fields. His experience includes numerous positions in Sales and Marketing, establishing Direct Sales, Service and Support companies in Europe and Australia, and managing Distributor Sales in over 55 countries worldwide. He has directly managed employees in Japan, China and most major EU countries.

# 2019 PRESS HIGHLIGHTS

READ MORE
Full articles available online at invivoscribe.com

**MAY 21, 2019**

## Invivoscribe Expanding Clinical Laboratory Network Space and Testing Capabilities in San Diego, Germany, Japan, and China. Offering Comprehensive Standardized Testing for All Hematologic Malignancies

Invivoscribe, Inc., a vertically integrated global company focused on Improving Lives with Precision Diagnostics®, today announces expansion of space and testing capabilities in their international network of accredited LabPMM® clinical laboratories. Additions to the test menu will include morphology, IHC, flow cytometry, as well as the latest multiparameter flow- (MPF), and NGS-based minimal residual disease (MRD) testing of primary specimens. These new services will further support rapid patient diagnosis, study subject enrollment, and study subject stratifications. They will be available by end of this year in San Diego followed by the laboratories in Japan, Germany and China.

**JUNE 19, 2019**

## Japan's MHLW Approves Invivoscribe's LeukoStrat CDx *FLT3* Mutation Assay as the CDx for Daiichi Sankyo's Quizartinib for Treatment of Relapsed/Refractory *FLT3*-ITD AML. Expands Use to Include Specimens Collected in EDTA

Invivoscribe is pleased to announce that on June 5th the Ministry of Health, Labor and Welfare (MHLW) approved our LeukoStrat® CDx *FLT3* Mutation Assay as the companion diagnostic for Daiichi Sankyo's Quizartinib for the treatment of *FLT3*-ITD positive relapse/ refractory acute myeloid leukemia (AML) patients in Japan. At the same time the Japanese MHLW added approval in use of EDTA collection tubes to the existing approval of heparin collection tubes used with this assay.

**DECEMBER 05, 2019**

## The European Commission Approves Astellas' XOSPATA® for Patients with Relapsed or Refractory Acute Myeloid Leukemia with a *FLT3* Mutation Detected by Validated Tests, including the Invivoscribe LeukoStrat CDx *FLT3* Mutation Assay

For twenty-five years, Invivoscribe has improved the quality of healthcare worldwide by providing high quality, standardized reagents, tests,

and bioinformatics tools to advance the field of precision medicine. The LeukoStrat® CDx *FLT3* Mutation Assay may now be used as an aid in the assessment of AML patients for treatment with XOSPATA® (gilteritinib) in Europe. *FLT3* mutation must be confirmed with a validated test, such as the LeukoStrat CDx *FLT3* Mutation Assay, which served as the companion diagnostic in the Phase 3 ADMIRAL trial resulting in approval of XOSPATA®.

Companion diagnostics play a key role in the development and approval of targeted drug therapies. The ability to screen for biomarkers in a patient population creates patient subsets which further enables drug developers in the design of novel therapeutics and management of clinical trials. Accordingly, the successful approval of a targeted therapy is highly dependent on the performance of the companion diagnostic.

# 2019 PRODUCT HIGHLIGHTS

**AUGUST 26, 2019**

## NOW AVAILABLE! MRD solutions for B- and T-cell clonality tracking

Minimal Residual Disease (MRD) is the presence of low levels of malignant cells that remain in a subject during or after treatment or when the patient is in remission. Detectable levels of cancer cells following treatment may suggest a higher probability of relapse.


BEST-IN-CLASS

# ASSAYS & REAGENTS

Invivoscribe provides a full range of standardized CE-marked *in vitro* diagnostic cGMP products for hematology-oncology, as well as RUO assays, analyte specific reagents (ASRs), and DNA & RNA controls.

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# Best-in-Class
# Assays & Reagents

Invivoscribe provides a full range of standardized CE-marked *in vitro* diagnostic cGMP products for hematology-oncology, as well as RUO assays, analyte specific reagents (ASRs), and DNA & RNA controls.

### Next-Generation Sequencing

NGS is a powerful, high-throughput DNA sequencing technology that allows for massively parallel sequencing of millions of DNA fragments in a single sequencing run. NGS is revolutionizing modern science and healthcare.

### ABI Fluorescence Detection

We exclusively offer a comprehensive selection of PCR-based assays for ABI fluorescence detection, including targeted *FLT3* ITD and TKD mutation assays, B- and T-cell clonality assays (based on EuroClonality/BIOMED-2 Concerted Action BMH4-CT98-3936), and translocation assays.

### Gel Detection

We exclusively offer a comprehensive selection of PCR-based assays for gel detection, including targeted *FLT3* ITD and TKD mutation assays, B- and T-cell clonality assays (based on EuroClonality/BIOMED-2 Concerted Action BMH4-CT98-3936), and translocation assays.

### Controls & Reagents

Invivoscribe offers an extensive range of General Purpose Reagents (GPRs) and Research Use Only (RUO) nucleic acid controls.

### Companion Diagnostics (CDx)

Invivoscribe is a Comprehensive Partner for Companion Diagnostic Development.

LabPMM®

# Clinical Lab Services

The Laboratory for Personalized Molecular Medicine® (LabPMM®) at Invivoscribe offer internationally standardized testing of novel and proprietary biomarkers that are critically important for patient care.

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Precision Diagnostics®

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online at invivoscribe.com

LabPMM®

# Clinical Lab Services

The Laboratory for Personalized Molecular Medicine® (LabPMM®) at Invivoscribe offer internationally standardized testing of novel and proprietary biomarkers that are critically important for patient care.

## NGS Cancer Panels

Cytogenetic identification of chromosome abnormalities has become essential for the clinical management of patients with leukemia and is currently used to help categorize patients into risk groups.

## MRD NGS Tests

LabPMM's MRD tests are NGS-based assays that can be used to detect clonal gene rearrangements identified at diagnosis within virtually all of the antigen receptor loci for both B- and T-cells.

## Targeted Genes

*FLT3* and *NPM1* assays are offered to detect targeted mutations.

## Clonality NGS Tests

The unique process of genetic rearrangements in the immunoglobulin (Ig) and T-cell receptor (TCR) gene loci during immune cell development and maturation generates a vast pool of genetically distinct cells.

## Companion Diagnostics (CDx) Tests

The fms related tyrosine kinase 3 (*FLT3*) is one of the most commonly mutated genes in acute myeloid leukemia (AML), occurring in approximately 30% of patients at the time of diagnosis.[1] Although generally associated with normal cytogenetics where patients have standard risk of relapse, *FLT3* mutations have also been identified in sub-groups of patients with chromosomal abnormalities that are associated with high risk of disease relapse.[2-3]

## Custom Assays

In response to the FDA announcing its intention to dramatically expand its regulatory oversight of laboratory developed tests (LDTs), Invivoscribe is partnering with laboratories worldwide to help facilitate the conversion of LDTs into FDA-cleared assays, as we know the barriers to bringing new assays online are often the availability of resources and the cost of validation.


1. Acute Myeloid Leukemia, Clinical Practice Guidelines in Oncology, (v.2.2014) National Comprehensive Cancer Network.
2. Lowenberg, B. et al. (1999) "Acute myeloid leukemia." N Engl J Med 341(14):1051-62.
3. Thiede, C. et al. (2002) "Analysis of FLT3-activating mutations in 979 patients with acute myelogenous leukemia: association with FAB and identification of subgroups with poor prognosis." Blood 99(12): 4326-35.

## STREAMLINED
# CDx PARNTERSHIPS

Companion Diagnostics represents a critical milestone in precision medicine and plays a pivotal role in the validation of targeted therapies. Traditional CDx development models involve multiple partners adding complexity and increasing risk which could affect drug approvals.

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# STREAMLINED CDx PARNTERSHIPS

Our Streamlined CDx™ program has demonstrated value, accelerating the international approvals of multiple new targeted therapies for multiple pharmaceutical companies.

## Product Development

### Design Controls

All biomarker assays & software developed under design controls.

### Bioinformatics Software

Comprehensive LymphoTrack® Dx clonality with bioinformatics software.

### Identify & Track

Multiple NGS gene panels that identify and track clinically-actionable biomarkers.

### Custom Development

Custom biomarker assay development.

### Complementary MRD Assays

Complementary MRD assays for all biomarkers – potential for surrogate endpoints per agency inputs.

## Manufacturing

### cGMP Compliant

US FDA/CDRH registered, EN ISO 13485:2016 certified manufacturing facility based in San Diego

### CE-marked IVDs & CDx Manufacturing

PMA companion diagnostics (CDx) for US and Japan, inclusion of CDx in ARTG Australia and >50 CE-IVDs (NGS assays include bioinformatics software).

### Assay Development

IUO & RUO assays, CE-marked IVDs, & CDx.

### Controls & Reagents

DNA / RNA controls, ASRs, GPRs, MRD controls & proficiency panels.

## LabPMM®

### Clinical Lab Experience

A dozen years of clinical reference lab experience.

### Internationally Standardized

Internationally standardized CDx and biomarker testing with internationally accredited labs serving the US, Europe, and Asia.

### Comprehensive Panels

Comprehensive LymphoTrack® clonality/MRD assays and NGS MyGene™ panels identify clinically-actionable biomarkers.

### Complementary MRD Assays

Complementary MRD assays for all biomarkers – potential for surrogate future endpoints per agency inputs.

### Worldwide Enrollment

Testing services have supported hundreds of enrollment sites worldwide.

## Global Regulatory Expertise

### Accredited & Proven

EN ISO 13485:2016 accredited. Experienced staff & proven Quality Management System.

### Registered Medical Device Establishment

Registered Medical Device Establishment with the US FDA, KFDA, Saudi Arabia, and the MHLW/PMDA.

### Multiple CDx Approvals

Multiple CDx approvals supporting various drugs: by the FDA (US), PMDA/MHLW (Japan), and TGA (Australia). CDx CE-marked IVD in the EU.

### CE-marked IVDs

>50 CE-marked IVDs available in the EU and select ROW markets; >60 tests included in the ARTG in Australia.

### Marketing Authorization Holder (MAH)

Marketing Authorization Holder (MAH) and national reimbursement for CDx in Japan.

**CONTACT**
Complete directory
online at invivoscribe.com

# GLOBAL DISTRIBUTOR NETWORK

A long and proud history of working with scientists, laboratorians and clinicians from around the world. We offer product support in over 160 countries. Our distributor partners are in locations around the globe.

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PERU
URUGUAY

# Table of Contents

# Background

## PCR & NGS-Based Assessment of Clonality in Hematologic Malignancies

Over its 25 year history, Invivoscribe has developed, manufactured, and commercialized the gold-standard molecular hematopathology assays and reagents for gel and capillary electrophoresis detection, and most recently, next-generation sequencing instruments. These standardized, cGMP manufactured assays and reagents were developed and validated using standardized workflow and optimized primer sets, reagents and controls.

A number of our products were developed in collaboration with studies conducted by the EuroClonality BIOMED-2 concerted action group; these capillary based products have provided reliable methods for clonality detection that have withstood the test of time.

We have never accepted the status quo, so our comprehensive menu of clonality assays continues to evolve. All of our NGS-based clonality assays were developed in-house together with accompanying bioinformatics software by our Invivoscribe R&D team. Developed under full ISO 13485 design control, these assays and bioinformatics software were designed to run on several next-generation sequencing platforms. These NGS-based assays are several generations ahead of capillary-based products.

Our comprehensive bioinformatics software not only provides critical information on the presence of clonality, but also identifies the sequence information required to track clones in subsequent samples.

The unique process of gene rearrangement that occurs within the immunoglobulin (*Ig*) and T-cell receptor (*TCR*) gene loci during immune cell development and maturation generates a vast pool of genetically distinct cells. The resulting diverse population of lymphocytes displays an astonishing number of diverse antigen receptors, each coded in the DNA by a unique sequence, and each displayed on the cell surface, or as antibodies in the blood unique to a given cell.[1,2] This diversity allows the adaptive immune system to carry out its role in protecting the human body by recognizing the infinite number of pathogens it might encounter during a lifetime.

In sum, lymphoid malignancies are characterized by size- and sequence-specific rearrangements within these loci, which result from the transformation and subsequent proliferation from a single cell. The associated cellular population typically shares one or more cell-specific or "clonal" antigen-receptor gene rearrangements. The detection of these clonal cells forms the basis for clonality assessment in leukemia, lymphoma, and hematologic disease. These methods can also be used to assess somatic hypermutation (SHM) and to study minimal residual disease (MRD).

Malignant cells that remain in the bone marrow following treatment are a major cause of disease relapse. MRD testing by NGS offers enhanced sensitivity and specificity (compared to MRD testing by flow cytometry), and allows residual cells to be identified at very low levels and monitored throughout the different stages of disease.

Invivoscribe can provide you with the necessary tools to accommodate your needs. From gel detection to NGS, we can help you accurately identify and track hematologic biomarkers.

For additional information on the detection methods available and the biomarkers offered, please refer to the respective product sections of this catalog.

## Immunoglobulin and T-Cell Receptor Gene Rearrangements and the Principle and Method of Clonality Testing

The adaptive vertebrate immune system produces a repertoire of immunoglobulin and T-cell receptor molecules using a relatively limited number of heritable germline gene segments. Somatic gene rearrangement is the fundamental mechanism used to generate different immunoglobulin and T-cell receptor molecules, each with unique binding specificity. Lymphocytes undergo gene rearrangements to assemble CDR3 coding regions that are unique in both size and DNA sequence. Since leukemias and lymphomas arise from the malignant transformation of a single cell, they share clonal rearrangement(s) of the antigen receptor genes. This is the basis for clonality testing.[3]

**References:**

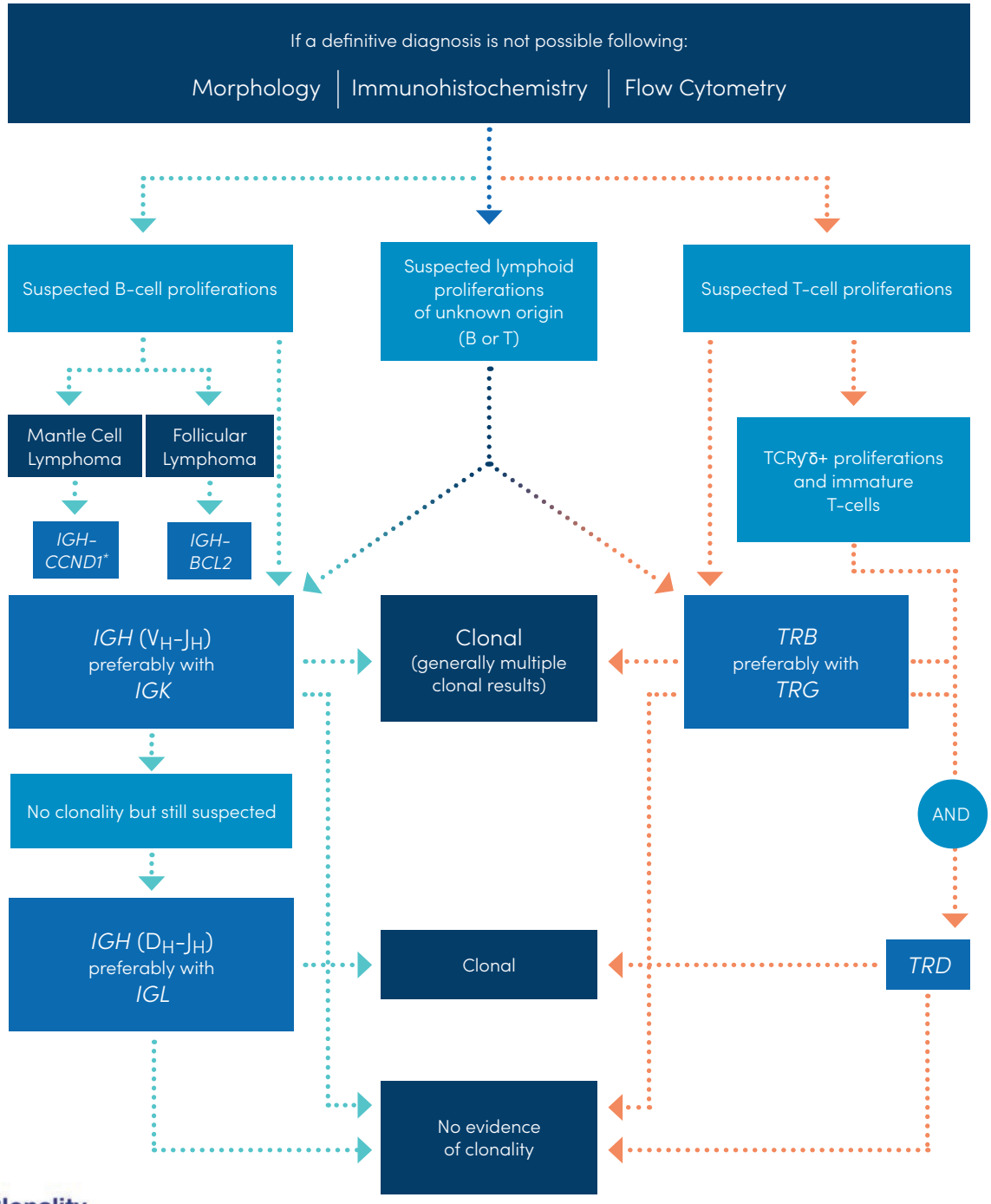
1. Tonegawa, S. Somatic Generation of Antibody Diversity. Nature 302:575-581 (1983)

2. Expression of T-cell receptor genes during early T-cell development. Immunol Cell Biol. 2008 Feb;86(2):166-74. Epub 2007 Oct 23.

3. Miller, J. E. (2013). Principle of Immunoglobulin and T cell Receptor Gene Rearrangement. In Cheng, L., Zhang, D., Eble, J. N. (Eds), *Molecular Genetic Pathology* (2nd Ed., Sections 30.2.7.13 and 30.2.7.18). pp825 – 856. New York, USA: Springer Science & Business Media.

# Test Algorithm for Suspect Lymphoproliferations

Developed in concert with the EuroClonality/BIOMED-2 group for PCR-based clonality assessment of suspected B- and T-cell lymphoproliferative disorders.




EuroClonality

*Previously known as *BCL1*/JH

**Results should be considered in the context of all available clinical, histological and immunophenotypic data.**

# LymphoTrack® Dx Assays

## Next-Generation Sequencing (NGS) CE IVD Assays

## Key Benefits

» One-step PCR for amplicon and library generation

» Identify, track, and assess mutation status of B- and T-cell gene rearrangements

» Sequence amplicons from any LymphoTrack Dx kit together

» Included bioinformatics software for easy analysis and interpretation

# LymphoTrack® Dx
Assays

## LymphoTrack Dx Assay kits are designed for the identification of gene rearrangements in hematologic samples utilizing next-generation sequencing (NGS) technologies.

### These assays take advantage of the wealth and depth of data generated by the Illumina® MiSeq®, Thermo Fisher Scientific® Ion PGM™ and Ion S5™ platforms.

The Invivoscribe NGS assays offer significant improvements over conventional fragment analysis of B- and T-cell gene rearrangements by providing detailed information regarding the DNA sequences, sequence frequency, and mutational status (*IGHV* Leader and *IGH* FR1 only) of each clonotype.

LymphoTrack Dx Assay kits are a complete solution. Kits contain ready-to-use indexed amplification master mixes, necessary controls, and complimentary bioinformatics software. As primers are designed with barcoded indices and adapters, sequencing libraries can be generated with a single PCR, streamlining the overall workflow, eliminating the need for a post-PCR ligation step, and reducing the potential for sample cross contamination.

The per sample testing costs can be reduced by pooling samples from different LymphoTrack Dx Assays into a single sequencing run. The included bioinformatics software will sort the complex NGS data for easy analysis and visualization of individual samples.

Detailed instructions for use are provided with all kits and the Invivoscribe technical support team is always available to answer your questions.

For more information, please visit www.invivoscribe.com

NOTICE:
The LymphoTrack Dx Assays are *in vitro* diagnostic products and are not available for sale or use within North America. For more information regarding the research use only reagents, please see the Next-Generation Sequencing - LymphoTrack section.

# LymphoTrack Dx *IGHV* Leader Somatic Hypermutation Assay

## Assay Description

The LymphoTrack Dx *IGHV* Leader Somatic Hypermutation Assay for the Illumina® MiSeq® is an *in vitro* diagnostic product intended for next-generation sequencing (NGS) based determination of the frequency distribution of *IGH* gene rearrangements, as well as the degree of somatic hypermutation (SHM) of rearranged genes in patients suspected of having lymphoproliferative disease. This assay aids in the identification of lymphoproliferative disorders as well as providing an aid in determining disease prognosis. If you would like to test for *IGHV* somatic hypermutation using the Thermo Fisher® Ion PGM™ or Ion S5™ platform, please refer to the LymphoTrack Dx *IGH* FR1 Assay (9-121-0007).

## Summary and Explanation of the Test

The NGS LymphoTrack Dx *IGHV* Leader Somatic Hypermutation Assay for the Illumina® MiSeq® represents a significant improvement over clonality assays using fragment analysis as it efficiently detects the majority of *IGH* gene rearrangements using a single multiplex master mix, identifies the DNA sequence specific for each clonal gene rearrangement, and assesses the somatic hypermutation rate of clonal samples in the same workflow.

The single multiplex master mixes target the Leader (VHL) and the joining (JH) gene regions of the *IGH* locus and are designed with Illumina® adapters and indices (8 included in Kit A and 24 included in the Panel). This allows for a one-step PCR reaction and pooling of amplicons from several different samples and targets into a single Illumina® MiSeq® run. No post-PCR ligation step is required.

The included LymphoTrack Dx Bioinformatics Software enables simplified analysis and visualization of individual sample data.

Positive (clonal positive, SHM negative), negative (polyclonal) and SHM (clonal positive, SHM positive) DNA controls are included in the kits.

## Background

The human immunoglobulin heavy chain (*IGH*) gene locus on chromosome 14 (14q32.3) includes 46-52 functional and 30 nonfunctional variable (VH) gene segments, 27 functional diversity (DH) gene segments, and 6 functional joining (JH) gene segments spread over 1,250 kilobases.

During B-cell development, genes encoding the *IGH* protein are assembled from multiple polymorphic gene segments that undergo rearrangements and selection, generating cell specific VH-DH-JH rearrangements that are unique in both length and sequence.

Since leukemias and lymphomas originate from the malignant transformation of individual lymphoid cells, all leukemias and lymphomas generally share one or more cell-specific or "clonal" antigen receptor gene rearrangements. Therefore, tests that detect *IGH* clonal rearrangements can be useful in the study of B- cell malignancies. An additional level of diversity is further generated in the antigen receptors by introducing point mutations in the variable regions, also named SHM. In instances where there is a high degree of SHM, there is the risk that primers located within the variable region will not be able to bind and clonal products will not amplify. In these cases, the leader primers located upstream of the variable region can be beneficial for the detection of clonal products, due to the conserved nature of the VHL region. In addition, the SHM rate of the entire variable gene can be determined using the VHL primers.

Determining the immunoglobulin variable heavy chain gene (*IGHV*) hypermutation rate is considered a gold standard for determining the prognosis of patients with chronic lymphocytic leukemia (CLL) and small lymphocytic lymphoma (SLL). In addition, NGS methods can improve disease stratification.

## Specimen Requirement

50 ng of high-quality genomic DNA.

## References

1. B Stamatopoulous et al., *Leukemia* 4:837-845 (2017).
2. F Davi et al., *Leukemia* 22: 212-214 (2008).
3. JE Miller et al., *Molecular Genetic Pathology* (2nd ed.). Springer Science & Business Media. 2013: 302.2.7.13 and 30.2.7.18.
4. P Ghia et al., *Leukemia* 21: 2-3 (2007).
5. P Ghia et al., *Blood* 105: 1678-1685 (2005).
6. S Tonegawa. *Nature* 302: 575-581 (1983).


Simplified representation of the immunoglobulin heavy chain (*IGH*) gene locus on chromosome 14. Depicted are the variable (VH) and downstream consensus joining (JH) region genes involved in rearrangements. Upstream of the variable gene segments, the leader sequence (VHL) is also depicted. Diversity region genes are not depicted.

# LymphoTrack® Dx

*IGHV* Leader Somatic Hypermutation Assay



## Reagents - MiSeq® Detection

### Kit A Components

| Master Mix Name | Index # |
|---|---|
| *IGH* Leader MiSeq 01 | A001 |
| *IGH* Leader MiSeq 02 | A002 |
| *IGH* Leader MiSeq 03 | A003 |
| *IGH* Leader MiSeq 04 | A004 |
| *IGH* Leader MiSeq 05 | A005 |
| *IGH* Leader MiSeq 06 | A006 |
| *IGH* Leader MiSeq 07 | A007 |
| *IGH* Leader MiSeq 08 | A008 |

| Controls |
|---|
| *IGH* SHM POS (+) Qty. 1 |
| *IGH* POS (+) Qty. 1 |
| NGS NEG (-) Qty. 1 |

### Panel Components (includes all master mixes from Kit A plus the items below)

| Master Mix Name | Index # | Master Mix Name | Index # |
|---|---|---|---|
| *IGH* Leader MiSeq 09 | A009 | *IGH* Leader MiSeq 18 | A018 |
| *IGH* Leader MiSeq 10 | A010 | *IGH* Leader MiSeq 19 | A019 |
| *IGH* Leader MiSeq 11 | A011 | *IGH* Leader MiSeq 20 | A020 |
| *IGH* Leader MiSeq 12 | A012 | *IGH* Leader MiSeq 21 | A021 |
| *IGH* Leader MiSeq 13 | A013 | *IGH* Leader MiSeq 22 | A022 |
| *IGH* Leader MiSeq 14 | A014 | *IGH* Leader MiSeq 23 | A023 |
| *IGH* Leader MiSeq 15 | A015 | *IGH* Leader MiSeq 25 | A025 |
| *IGH* Leader MiSeq 16 | A016 | *IGH* Leader MiSeq 27 | A027 |

| Controls |
|---|
| *IGH* SHM POS (+) Qty. 3 |
| *IGH* POS (+) Qty. 3 |
| NGS NEG (-) Qty. 3 |

| Rank | Sequence | Length | Merge count | V-gene | J-gene | % Total reads | Cumulative % | Mutation rate partial V-gene (%) | In-frame (Y/N) | No stop codon (Y/N) | V-coverage |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | TTCTCGTGGTG | 455 | 29603 | *IGHV*4-59_08 | *IGHJ*4_02 | 9.93 | 9.93 | 11.26 | Y | Y | 98.63 |
| 2 | CTCGCCCTCCT | 463 | 205 | *IGHV*5-51_01 | *IGHJ*4_02 | 0.07 | 9.99 | 0.00 | Y | Y | 99.66 |
| 3 | GGTTTTCCTTG | 484 | 201 | *IGHV*3-7_01 | *IGHJ*4_02 | 0.07 | 10.06 | 7.77 | Y | Y | 100.00 |
| 4 | CTCGCCCTCCT | 463 | 185 | *IGHV*5-51_01 | *IGHJ*5_02 | 0.06 | 10.12 | 6.08 | Y | Y | 99.32 |
| 5 | CTCGCCCTCCT | 469 | 170 | *IGHV*5-51_01 | *IGHJ*4_02 | 0.06 | 10.18 | 0.00 | Y | Y | 99.32 |
| 6 | CTCGCCCTCCT | 466 | 160 | *IGHV*5-51_01 | *IGHJ*4_02 | 0.05 | 10.23 | 0.00 | Y | Y | 99.66 |
| 7 | CTGCTGCTGAC | 460 | 159 | *IGHV*2-5_10 | *IGHJ*5_02 | 0.05 | 10.29 | 8.08 | Y | Y | 97.64 |
| 8 | GGTTTTCCTTG | 493 | 156 | *IGHV*3-48_02 | *IGHJ*6_02 | 0.05 | 10.34 | 3.72 | Y | Y | 98.99 |
| 9 | CTCGCCCTCCT | 334 | 153 | *IGHV*5-51_02 | *IGHJ*2_01 | 0.05 | 10.39 | 3.72 | Y | N | 27.70 |
| 10 | CTCGCCCTCCT | 334 | 152 | *IGHV*5-51_02 | *IGHJ*2_01 | 0.05 | 10.44 | 3.38 | Y | N | 26.01 |

**Example Data.** The top 10 sequences from a read summary generated by the LymphoTrack Dx Software - MiSeq® with the SHM mutation rate and predictions pertaining to whether a sequence is in-frame or contains a premature stop codon are depicted. To learn more about the LymphoTrack Dx software, please refer to the LymphoTrack Dx Bioinformatics Software section.

## Ordering Information

| Catalog # | Products | Quantity Components |
|---|---|---|
| 9-121-0059 | LymphoTrack® Dx *IGHV* Leader Somatic Hypermutation Assay Kit A - MiSeq® | 8 indices - 5 sequencing reactions each |
| 9-121-0069 | LymphoTrack® Dx *IGHV* Leader Somatic Hypermutation Assay Panel - MiSeq® | 24 indices - 5 sequencing reactions each |
| 9-500-0009 | LymphoTrack® Dx Software - MiSeq® | 1 CD complimentary with purchase |

CE IVD These products are CE-IVD assays for *in vitro* diagnostic use.

# LymphoTrack Dx *IGH* FR1/2/3 Assays

## Assay Description

**LymphoTrack Dx *IGH* FR1 Assays**
The LymphoTrack Dx *IGH* FR1 Assay for the Illumina MiSeq® or Thermo Fisher Scientific® Ion S5™ and Ion PGM™ is an *in vitro* diagnostic product intended for next-generation sequencing (NGS) based determination of the frequency distribution of *IGH* gene rearrangements as well as the degree of somatic hypermutation of rearranged genes in patients suspected with having lymphoproliferative disease. This assay aids in the identification of lymphoproliferative disorders as well as providing an aid in determining disease prognosis.

**LymphoTrack Dx *IGH* FR1/2/3 Assays**
The LymphoTrack Dx *IGH* FR1 Assay for the Illumina MiSeq® or Thermo Fisher Scientific® Ion S5™ and Ion PGM™ is an *in vitro* diagnostic product intended for next-generation sequencing (NGS) based determination of the frequency distribution of *IGH* gene rearrangements as well as the degree of somatic hypermutation of rearranged genes in patients suspected with having lymphoproliferative disease. This assay aids in the identification of lymphoproliferative disorders as well as providing an aid in determining disease prognosis.

This LymphoTrack Dx *IGH* FR2 Assay is an *in vitro* diagnostic product intended for next-generation sequencing (NGS) for the Illumina MiSeq® or Thermo Fisher Scientific® Ion S5™ and Ion PGM™ instruments. The assay will determine the frequency distribution of *IGH* $V_H$-$J_H$ gene rearrangements in patients suspected with having lymphoproliferative disease. This assay aids in the identification of lymphoproliferative disorders.

The LymphoTrack Dx *IGH* FR3 Assay is an *in vitro* diagnostic product intended for next-generation sequencing (NGS) for the Illumina MiSeq® or Thermo Fisher Scientific® Ion S5™ and Ion PGM™ instruments. The assay will determine the frequency distribution of *IGH* $V_H$-$J_H$ gene rearrangements in patients suspected with having lymphoproliferative disease. This assay aids in the identification of lymphoproliferative disorders.

## Summary and Explanation of the Test

The LymphoTrack Dx *IGH* Assays represent a significant improvement over conventional clonality assessment methods utilizing fragment analysis by providing four important and complementary uses in a single workflow:

1. Detection of clonal populations.
2. Identification of sequence information required to track clonal rearrangements in subsequent samples.
3. The LymphoTrack Dx *IGH* framework 1 FR1 master mixes provide the degree of SHM in the immunoglobulin variable heavy chain (*IGHV*) gene locus.
4. The ability to track sequences in subsequent samples with the Invivoscribe LymphoTrack MRD* Software. For more information, please refer to page 52 and 53.

These assays utilize a single multiplex master mix to target each conserved *IGH* Framework Region (FR1, FR2, and FR3) within the VH and the JH regions described in lymphoid malignancies. The included primers are designed with Illumina® or Thermo Fisher Scientific adapters and indices (8-24 and 12, respectively).

This allows up to 24 samples on MiSeq® and 12 samples on Ion PGM™ and Ion S5™ to be sequenced at the same time with any of the individual FRs.

In addition, amplicons generated with different FR master mixes or Invivoscribe LymphoTrack Dx kits (such as *IGK* or *TRG*) can be pooled together in the same sequencing library to reduce testing costs. The associated LymphoTrack Dx Software provides interpretation of the data via a simple and streamlined method of analysis and visualization. By following the guidelines provided in the instructions for use, samples can be interpreted for evidence of clonality and SHM rates.

Positive clonal (SHM negative) and negative polyclonal DNA controls are included in kits. A clonal SHM positive control can be purchased separately (cat#: 4-088-0008).

## Background

The human immunoglobulin heavy chain (*IGH*) gene locus on chromosome 14 (14q32.3) includes 46-52 functional and 30 non-functional variable (VH), 27 functional diversity (DH), and 6 functional joining (JH) gene segments. The VH gene segments can be further broken down into three conserved frameworks (FR) and three variable complementarity-determining regions (CDRs).

During development of lymphoid cells, antigen receptor genes undergo somatic gene rearrangements. Specifically during B-cell development, *IGH* molecules are assembled from multiple polymorphic gene segments that undergo rearrangements generating VH-DH-JH combinations unique in both length and sequence. Since leukemias and lymphomas originate from the malignant transformation of individual lymphoid cells, all leukemias and lymphomas generally share one or more cell-specific or "clonal" antigen receptor gene rearrangements.

In addition, the *IGHV* hypermutation status obtained with the LymphoTrack Dx *IGH* FR1 master mixes, provides important prognostic information for patients with chronic lymphocytic leukemia (CLL) and small lymphocytic lymphoma (SLL). The SHM rate has been shown to have clinical relevance for CLL, as there is a clear distinction in the median survival of patients with and without SHM.[1]

## Specimen Requirement

50 ng of high-quality genomic DNA.

## References

1. P Ghia et al., Blood 105: 1678-1685 (2005).



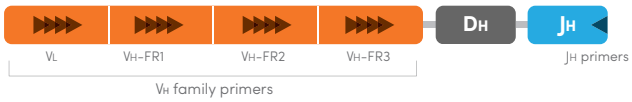
*MRD Software can be used to track sequences generated by either LymphoTrack Assays - MiSeq® or Ion S5/PGM™. MRD applications are for Research Use Only. To obtain a copy, please contact your local distributor or send an e-mail to customerservice@invivoscribe.com

# LymphoTrack® Dx

*IGH* Assay

Next-Generation Sequencing (NGS) | CE-IVD Assays

## Simplified Representation of the *IGH* Gene




Simplified depiction of variable (VH) and downstream consensus joining (JH) region genes involved in gene rearrangements.

## Reagents - MiSeq® Detection

The LymphoTrack Dx *IGH* FR1/2/3 Assays contain components from respective individual FR kit A's or panels.

| LymphoTrack Dx *IGH* FR1 Components | | | LymphoTrack Dx *IGH* FR2 Components | | | LymphoTrack Dx *IGH* FR3 Components | | |
|---|---|---|---|---|---|---|---|---|
| Master Mix Name | Index # | | Master Mix Name | Index # | | Master Mix Name | Index # | |
| *IGH* FR1 MiSeq 01 | A001 | KIT A | *IGH* FR2 MiSeq 01 | A001 | KIT A | *IGH* FR3 MiSeq 01 | A001 | KIT A |
| *IGH* FR1 MiSeq 02 | A002 | | *IGH* FR2 MiSeq 02 | A002 | | *IGH* FR3 MiSeq 02 | A002 | |
| *IGH* FR1 MiSeq 03 | A003 | | *IGH* FR2 MiSeq 03 | A003 | | *IGH* FR3 MiSeq 03 | A003 | |
| *IGH* FR1 MiSeq 04 | A004 | | *IGH* FR2 MiSeq 04 | A004 | | *IGH* FR3 MiSeq 04 | A004 | |
| *IGH* FR1 MiSeq 05 | A005 | | *IGH* FR2 MiSeq 05 | A005 | | *IGH* FR3 MiSeq 05 | A005 | |
| *IGH* FR1 MiSeq 06 | A006 | | *IGH* FR2 MiSeq 06 | A006 | | *IGH* FR3 MiSeq 06 | A006 | |
| *IGH* FR1 MiSeq 07 | A007 | | *IGH* FR2 MiSeq 07 | A007 | | *IGH* FR3 MiSeq 07 | A007 | |
| *IGH* FR1 MiSeq 08 | A008 | | *IGH* FR2 MiSeq 08 | A008 | | *IGH* FR3 MiSeq 08 | A008 | |
| *IGH* FR1 MiSeq 09 | A009 | PANEL | *IGH* FR2 MiSeq 09 | A009 | PANEL | *IGH* FR3 MiSeq 09 | A009 | PANEL |
| *IGH* FR1 MiSeq 10 | A010 | | *IGH* FR2 MiSeq 10 | A010 | | *IGH* FR3 MiSeq 10 | A010 | |
| *IGH* FR1 MiSeq 11 | A011 | | *IGH* FR2 MiSeq 11 | A011 | | *IGH* FR3 MiSeq 11 | A011 | |
| *IGH* FR1 MiSeq 12 | A012 | | *IGH* FR2 MiSeq 12 | A012 | | *IGH* FR3 MiSeq 12 | A012 | |
| *IGH* FR1 MiSeq 13 | A013 | | *IGH* FR2 MiSeq 13 | A013 | | *IGH* FR3 MiSeq 13 | A013 | |
| *IGH* FR1 MiSeq 14 | A014 | | *IGH* FR2 MiSeq 14 | A014 | | *IGH* FR3 MiSeq 14 | A014 | |
| *IGH* FR1 MiSeq 15 | A015 | | *IGH* FR2 MiSeq 15 | A015 | | *IGH* FR3 MiSeq 15 | A015 | |
| *IGH* FR1 MiSeq 16 | A016 | | *IGH* FR2 MiSeq 16 | A016 | | *IGH* FR3 MiSeq 16 | A016 | |
| *IGH* FR1 MiSeq 18 | A018 | | *IGH* FR2 MiSeq 18 | A018 | | *IGH* FR3 MiSeq 18 | A018 | |
| *IGH* FR1 MiSeq 19 | A019 | | *IGH* FR2 MiSeq 19 | A019 | | *IGH* FR3 MiSeq 19 | A019 | |
| *IGH* FR1 MiSeq 20 | A020 | | *IGH* FR2 MiSeq 20 | A020 | | *IGH* FR3 MiSeq 20 | A020 | |
| *IGH* FR1 MiSeq 21 | A021 | | *IGH* FR2 MiSeq 21 | A021 | | *IGH* FR3 MiSeq 21 | A021 | |
| *IGH* FR1 MiSeq 22 | A022 | | *IGH* FR2 MiSeq 22 | A022 | | *IGH* FR3 MiSeq 22 | A022 | |
| *IGH* FR1 MiSeq 23 | A023 | | *IGH* FR2 MiSeq 23 | A023 | | *IGH* FR3 MiSeq 23 | A023 | |
| *IGH* FR1 MiSeq 25 | A025 | | *IGH* FR2 MiSeq 25 | A025 | | *IGH* FR3 MiSeq 25 | A025 | |
| *IGH* FR1 MiSeq 27 | A027 | | *IGH* FR2 MiSeq 27 | A027 | | *IGH* FR3 MiSeq 27 | A027 | |

Kit A's contain indices *IGH* FRX A001 to A008. Panels contain all master mixes listed above.

| Controls in Individual FR (1, 2, or 3) Kit A's | Controls in Individual FR (1, 2, or 3) Panels | Controls in Combo FR 1/2/3 Kit A | Controls in Combo FR 1/2/3 Panel |
|---|---|---|---|
| *IGH* POS (+) Qty. 1 | *IGH* POS (+) Qty 3 | *IGH* POS (+) Qty. 2 | *IGH* POS (+) Qty. 6 |
| NGS NEG (-) Qty. 1 | NGS NEG (-) Qty 3 | NGS NEG (-) Qty. 2 | NGS NEG (-) Qty. 6 |

# LymphoTrack Dx *IGH* FR1/2/3 Assays continued

## Reagents - Ion S5™ / PGM™ Detection

The LymphoTrack Dx *IGH* FR1/2/3 Assays contain components from respective individual FR Assays.

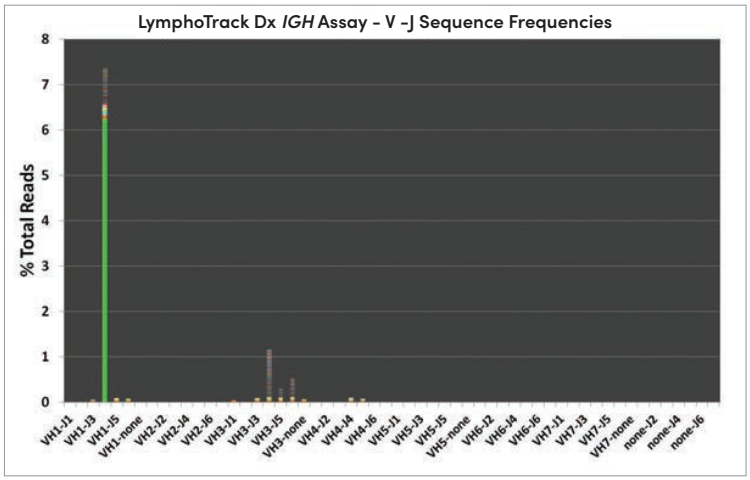
| LymphoTrack Dx *IGH* FR1 Components | | LymphoTrack Dx *IGH* FR2 Components | | LymphoTrack Dx *IGH* FR3 Components | |
|---|---|---|---|---|---|
| Master Mix Name | Index # | Master Mix Name | Index # | Master Mix Name | Index # |
| *IGH* FR1 Ion S5/PGM 01 | IonXpress_001 | *IGH* FR2 Ion S5/PGM 01 | IonXpress_001 | *IGH* FR3 Ion S5/PGM 01 | IonXpress_001 |
| *IGH* FR1 Ion S5/PGM 02 | IonXpress_002 | *IGH* FR2 Ion S5/PGM 02 | IonXpress_002 | *IGH* FR3 Ion S5/PGM 02 | IonXpress_002 |
| *IGH* FR1 Ion S5/PGM 03 | IonXpress_003 | *IGH* FR2 Ion S5/PGM 03 | IonXpress_003 | *IGH* FR3 Ion S5/PGM 03 | IonXpress_003 |
| *IGH* FR1 Ion S5/PGM 04 | IonXpress_004 | *IGH* FR2 Ion S5/PGM 04 | IonXpress_004 | *IGH* FR3 Ion S5/PGM 04 | IonXpress_004 |
| *IGH* FR1 Ion S5/PGM 07 | IonXpress_007 | *IGH* FR2 Ion S5/PGM 07 | IonXpress_007 | *IGH* FR3 Ion S5/PGM 07 | IonXpress_007 |
| *IGH* FR1 Ion S5/PGM 08 | IonXpress_008 | *IGH* FR2 Ion S5/PGM 08 | IonXpress_008 | *IGH* FR3 Ion S5/PGM 08 | IonXpress_008 |
| *IGH* FR1 Ion S5/PGM 09 | IonXpress_009 | *IGH* FR2 Ion S5/PGM 09 | IonXpress_009 | *IGH* FR3 Ion S5/PGM 09 | IonXpress_009 |
| *IGH* FR1 Ion S5/PGM 10 | IonXpress_010 | *IGH* FR2 Ion S5/PGM 10 | IonXpress_010 | *IGH* FR3 Ion S5/PGM 10 | IonXpress_010 |
| *IGH* FR1 Ion S5/PGM 11 | IonXpress_011 | *IGH* FR2 Ion S5/PGM 11 | IonXpress_011 | *IGH* FR3 Ion S5/PGM 11 | IonXpress_011 |
| *IGH* FR1 Ion S5/PGM 12 | IonXpress_012 | *IGH* FR2 Ion S5/PGM 12 | IonXpress_012 | *IGH* FR3 Ion S5/PGM 12 | IonXpress_012 |
| *IGH* FR1 Ion S5/PGM 13 | IonXpress_013 | *IGH* FR2 Ion S5/PGM 13 | IonXpress_013 | *IGH* FR3 Ion S5/PGM 13 | IonXpress_013 |
| *IGH* FR1 Ion S5/PGM 14 | IonXpress_014 | *IGH* FR2 Ion S5/PGM 14 | IonXpress_014 | *IGH* FR3 Ion S5/PGM 14 | IonXpress_014 |

| Controls in Individual FR (1,2, or 3) Kits | Controls in FR 1/2/3 Kit |
|---|---|
| *IGH* POS (+) Qty. 2 | *IGH* POS (+) Qty. 4 |
| NGS NEG (-) Qty. 2 | NGS NEG (-) Qty. 4 |

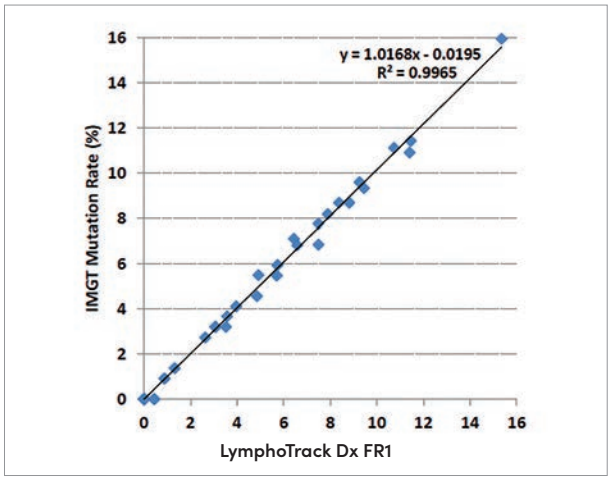
# LymphoTrack® Dx

*IGH* Assay

Next-Generation Sequencing (NGS) | CE-IVD Assays




**V-J Sequence Frequencies.** The LymphoTrack Dx Software provides a stacked bar graph depicting the relative frequencies of the 200 most prevalent VH-JH rearrangements identified in a sample. To learn more about the LymphoTrack Dx software, please refer to the LymphoTrack Dx Bioinformatics Software section.




Comparison of SHM Analysis Methods. The SHM rate of 51 CLL samples was determined by the LymphoTrack Dx *IGH* FR1 Assay – MiSeq® and analyzed with both the LymphoTrack Dx Software - MiSeq® and IMGT analysis.

## Ordering Information

| Catalog # | Products | Quantity Components |
|---|---|---|
| 9-121-0129 | LymphoTrack® Dx *IGH* FR1/2/3 Assay Kit A - MiSeq® | 8 + 8 + 8 indices - 5 sequencing reactions each |
| 9-121-0139 | LymphoTrack® Dx *IGH* FR1/2/3 Assay Panel - MiSeq® | 24 + 24 + 24 indices - 5 sequencing reactions each |
| 9-121-0009 | LymphoTrack® Dx *IGH* FR1 Assay Kit A - MiSeq® | 8 indices - 5 sequencing reactions each |
| 9-121-0039 | LymphoTrack® Dx *IGH* FR1 Assay Panel - MiSeq® | 24 indices - 5 sequencing reactions each |
| 9-121-0089 | LymphoTrack® Dx *IGH* FR2 Assay Kit A - MiSeq® | 8 indices - 5 sequencing reactions each |
| 9-121-0099 | LymphoTrack® Dx *IGH* FR2 Assay Panel - MiSeq® | 24 indices - 5 sequencing reactions each |
| 9-121-0109 | LymphoTrack® Dx *IGH* FR3 Assay Kit A - MiSeq® | 8 indices - 5 sequencing reactions each |
| 9-121-0119 | LymphoTrack® Dx *IGH* FR3 Assay Panel - MiSeq® | 24 indices - 5 sequencing reactions each |
| 9-500-0009 | LymphoTrack® Dx Software - MiSeq® | 1 CD complimentary with purchase |
| 9-121-0057 | LymphoTrack® Dx *IGH* FR1/2/3 Assay - S5/PGM™ | 12 + 12 + 12 indices - 5 sequencing reactions each |
| 9-121-0007 | LymphoTrack® Dx *IGH* FR1 Assay - S5/PGM™ | 12 indices - 5 sequencing reactions each |
| 9-121-0037 | LymphoTrack® Dx *IGH* FR2 Assay - S5/PGM™ | 12 indices - 5 sequencing reactions each |
| 9-121-0047 | LymphoTrack® Dx *IGH* FR3 Assay - S5/PGM™ | 12 indices - 5 sequencing reactions each |
| 9-500-0007 | LymphoTrack® Dx Software - S5/PGM™ | 1 CD complimentary with purchase |

CE IVD These products are CE-IVD assays for *in vitro* diagnostic use.

# LymphoTrack Dx *IGK* Assay

## Assay Description

The LymphoTrack Dx *IGK* Assays for the Illumina® MiSeq®, or Thermo Fisher Scientific® Ion PGM™ and Ion S5™ instruments are *in vitro* diagnostic products intended for next-generation sequencing (NGS) based determination of the frequency distribution of *IGK* gene rearrangements in patients suspected of having lymphoproliferative disease. These assays aid in the identification of lymphoproliferative disorders.

## Summary and Explanation of the Test

In contrast to the IdentiClone® fragment analysis assays for clonality that utilize two master mixes, these NGS assays contain a single multiplex master mix to target conserved regions of the *IGK* gene locus described in lymphoid malignancies, thereby, reducing sample DNA requirements and simplifying the testing workflow. The LymphoTrack Dx *IGK* master mix primers are also designed with Illumina® or Thermo Fisher Scientific adapters and up to 24 different indices. This allows amplicons generated from different indexed *IGK* master mixes to be pooled into a single library for loading onto one MiSeq® flow cell, Ion PGM™ or Ion S5™ chips.

The associated LymphoTrack Dx Software is capable of sorting complex NGS data by gene target, providing users the ability to reduce per sample testing costs by sequencing amplicons from any LymphoTrack Dx Assay (*e.g. IGH, IGK, TRB, TRG*) at the same time. In addition, the LymphoTrack Dx Software provides an easy and streamlined method for visualization of data and guidelines provided in the instructions for use allow samples to be interpreted for evidence or no evidence of clonality.

Positive clonal and negative polyclonal DNA controls are included in kits.

## Background

The LymphoTrack Dx *IGK* Assays represent a significant improvement over existing fragment analysis clonality assays by providing two important and complementary uses:

1. Detection of initial clonal populations.
2. Identification of sequence information required to track clonal rearrangements in subsequent samples.

The human immunoglobulin kappa (*IGK*) gene locus on chromosome 2 (2p11.2) includes 76 V (variable) region genes spanning 7 subgroups and 5 J (joining) region gene segments upstream of the (Cκ) region.

During lymphoid cell development, antigen receptor genes undergo somatic gene rearrangements. Specifically, during B-cell development, genes encoding *IGK* molecules are assembled from multiple polymorphic gene segments that generate V-J combinations unique in both length and sequence.

In addition, the kappa deleting element ($K_{de}$), approximately 24 kb downstream of the Jκ-Cκ region can also rearrange with Vκ gene segments and the isolated recombination signal sequence in the Jκ-Cκ intron (Jκ-Cκ INTR).

Since leukemias and lymphomas originate from the malignant transformation of individual lymphoid cells, all leukemias and lymphomas generally share one or more cell-specific or "clonal" antigen receptor gene rearrangements. Therefore, tests that detect *IGK* clonal rearrangements can be useful in the study of B- cell malignancies and complement *IGH* testing, as the *IGK* receptor is less susceptible to somatic mutations.

## Specimen Requirement

50 ng of high-quality genomic DNA.

## References

1. EP Rock et al., J *Exp Med* 179 (1): 323-8 (1994).
2. JE Miller et al., *Molecular Diagnostic* 4 (2): 102-117(1999).

## Simplified Representation of the *IGK* Gene


Depicted are the variable region (Vκ) or variable intragenic Jκ-Cκ intron (Jκ-Cκ INTR) genes involved in *IGK* gene rearrangements in addition to the downstream consensus joining region genes (Jκ) or kappa deleting element ($K_{de}$).

# LymphoTrack® Dx

*IGK* Assay

Next-Generation Sequencing (NGS) | CE-IVD Assays

## Reagents - MiSeq® Detection

### Kit A Components

| Master Mix Name | Index # |
|---|---|
| *IGK* MiSeq 01 | A001 |
| *IGK* MiSeq 02 | A002 |
| *IGK* MiSeq 03 | A003 |
| *IGK* MiSeq 04 | A004 |
| *IGK* MiSeq 05 | A005 |
| *IGK* MiSeq 06 | A006 |
| *IGK* MiSeq 07 | A007 |
| *IGK* MiSeq 08 | A008 |

| Controls | |
|---|---|
| *IGK* POS (+) Qty. 1 | NGS NEG (-) Qty. 1 |

### Panel Components (includes all master mixes from Kit A plus the items below)

| Master Mix Name | Index # | Master Mix Name | Index # |
|---|---|---|---|
| *IGK* MiSeq 09 | A009 | *IGK* MiSeq 18 | A018 |
| *IGK* MiSeq 10 | A010 | *IGK* MiSeq 19 | A019 |
| *IGK* MiSeq 11 | A011 | *IGK* MiSeq 20 | A020 |
| *IGK* MiSeq 12 | A012 | *IGK* MiSeq 21 | A021 |
| *IGK* MiSeq 13 | A013 | *IGK* MiSeq 22 | A022 |
| *IGK* MiSeq 14 | A014 | *IGK* MiSeq 23 | A023 |
| *IGK* MiSeq 15 | A015 | *IGK* MiSeq 25 | A025 |
| *IGK* MiSeq 16 | A016 | *IGK* MiSeq 27 | A027 |

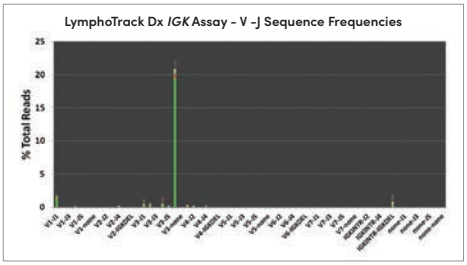
| Controls | |
|---|---|
| *IGK* POS (+) Qty. 3 | NGS NEG (-) Qty. 3 |

## Reagents - Ion S5™/PGM™ Detection

### Assay Components

| Master Mix Name | Index # | Master Mix Name | Index # |
|---|---|---|---|
| *IGK* S5/PGM 01 | IonXpress_001 | *IGK* S5/PGM 11 | IonXpress_011 |
| *IGK* S5/PGM 02 | IonXpress_002 | *IGK* S5/PGM 12 | IonXpress_012 |
| *IGK* S5/PGM 04 | IonXpress_004 | *IGK* S5/PGM 13 | IonXpress_013 |
| *IGK* S5/PGM 08 | IonXpress_008 | *IGK* S5/PGM 14 | IonXpress_014 |
| *IGK* S5/PGM 09 | IonXpress_009 | *IGK* S5/PGM 16 | IonXpress_016 |
| *IGK* S5/PGM 010 | IonXpress_010 | *IGK* S5/PGM 17 | IonXpress_017 |

| Controls | |
|---|---|
| *IGK* POS (+) Qty. 2 | NGS NEG (-) Qty. 2 |




**V-J Sequence Frequencies.** The LymphoTrack Dx Software provides a stacked bar graph depicting the relative frequencies for the most prevalent rearrangements identified in a sample. To learn more about the LymphoTrack Dx software, please refer to the LymphoTrack Dx Bioinformatics Software section.

## Ordering Information

| Catalog # | Products | Quantity Components |
|---|---|---|
| 9-122-0009 | LymphoTrack® Dx *IGK* Assay Kit A - MiSeq® | 8 indices - 5 sequencing reactions each |
| 9-122-0019 | LymphoTrack® Dx *IGK* Assay Panel - MiSeq® | 24 indices - 5 sequencing reactions each |
| 9-500-0009 | LymphoTrack® Dx Software - MiSeq® | 1 CD complimentary with purchase |
| 9-122-0007 | LymphoTrack® Dx *IGK* Assay - S5/PGM™ | 12 indices - 5 sequencing reactions each |
| 9-500-0007 | LymphoTrack® Dx Software - S5/PGM™ | 1 CD complimentary with purchase |

CE IVD These products are CE-IVD assays for *in vitro* diagnostic use.

# LymphoTrack Dx *TRG* Assay

## Assay Description

The LymphoTrack Dx *TRG* Assays for the Illumina® MiSeq®, Thermo Fisher Scientific® Ion PGM™ or Ion S5™ instruments are *in vitro* diagnostic products intended for next-generation sequencing (NGS) based determination of the frequency distribution of *TRG* gene rearrangements in patients suspected with having lymphoproliferative disease. These assays aid in the identification of lymphoproliferative disorders.

## Summary and Explanation of the Test

These assays utilize a single multiplex master mix to target conserved V and J regions of the human T-cell receptor gamma (*TRG*) gene that are described in lymphoid malignancies. Primers are designed with Illumina® or Thermo Fisher Scientific adapters and up to 24 different indices; thereby allowing amplicons generated from different *TRG* master mixes to be pooled together for sequencing on a single MiSeq® flow cell, or Ion PGM™ or Ion S5™ chip.

The associated LymphoTrack Dx Software sorts complex NGS data by gene target, providing users the ability to reduce per sample testing costs by sequencing amplicons generated with any LymphoTrack Dx Assay (*e.g. IGH, IGK, TRB, TRG*) at the same time. In addition, the LymphoTrack Dx Software provides an easy and streamlined method for data visualization and guidelines provided in the instructions for use allow samples to be interpreted for evidence or no evidence of clonality.

Positive clonal and negative polyclonal DNA controls are included in kits.

## Background

The LymphoTrack Dx *TRG* Assays represent a significant improvement over existing fragment analysis clonality assays by providing two important and complementary uses:

1. Detection of initial clonal populations.
2. Identification of sequence information required to track clonal rearrangements in subsequent samples.

The *TRG* gene locus on chromosome 7 (7q14) includes 14 V (variable region) genes (Group I, II, III, and IV), 5 J (joining region) gene segments, and 2 C (constant region) genes spread over 200 kilobases.

During development of lymphoid cells, antigen receptor genes undergo somatic gene rearrangements. Specifically during T-cell development, genes encoding *TRG* molecules are assembled from multiple polymorphic gene segments that undergo rearrangement generating V-J combinations unique in both length and sequence. Since leukemias and lymphomas originate from the malignant transformation of individual lymphoid cells, all leukemias and lymphomas generally share one or more cell-specific or "clonal" antigen receptor gene rearrangement. Therefore, tests that detect *TRG* clonal rearrangements can be useful in the study of B- and T-cell malignancies.

## Specimen Requirement

50 ng of high-quality genomic DNA.

## References

1. S Tonegawa. *Nature* 302: 575-581 (1983).
2. JE Miller et al., *Molecular Genetic Pathology* (2nd ed.). Springer Science & Business Media. 2013: 30.2.7.13.

## Simplified Representation of the *TRG* Gene


Depicted are the variable region (V) genes and downstream consensus joining region genes (J) that are involved in *TRG* gene rearrangements.

# LymphoTrack® Dx

*TRG* Assay



## Reagents - MiSeq® Detection

### Kit A Components

| Master Mix Name | Index # |
|---|---|
| *TRG* MiSeq 01 | A001 |
| *TRG* MiSeq 02 | A002 |
| *TRG* MiSeq 03 | A003 |
| *TRG* MiSeq04 | A004 |
| *TRG* MiSeq 05 | A005 |
| *TRG* MiSeq 06 | A006 |
| *TRG* MiSeq 07 | A007 |
| *TRG* MiSeq 08 | A008 |
| Controls | |
| *TRG* POS (+) Qty. 1 | NGS NEG (-) Qty. 1 |

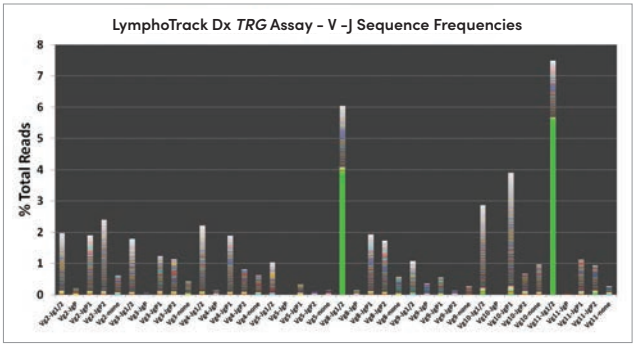
### Panel Components (includes all master mixes from Kit A plus the items below)

| Master Mix Name | Index # | Master Mix Name | Index # |
|---|---|---|---|
| *TRG* MiSeq 09 | A009 | *TRG* MiSeq 18 | A018 |
| *TRG* MiSeq 10 | A010 | *TRG* MiSeq 19 | A019 |
| *TRG* MiSeq 11 | A011 | *TRG* MiSeq 20 | A020 |
| *TRG* MiSeq 12 | A012 | *TRG* MiSeq 21 | A021 |
| *TRG* MiSeq 13 | A013 | *TRG* MiSeq 22 | A022 |
| *TRG* MiSeq 14 | A014 | *TRG* MiSeq 23 | A023 |
| *TRG* MiSeq 15 | A015 | *TRG* MiSeq 25 | A025 |
| *TRG* MiSeq 16 | A016 | *TRG* MiSeq 27 | A027 |
| Controls | | | |
| *TRG* POS (+) Qty. 3 | | NGS NEG (-) Qty. 3 | |

## Reagents - Ion S5™/PGM™ Detection

### Assay components

| Master Mix Name | Index # | Master Mix Name | Index # | Master Mix Name | Index # |
|---|---|---|---|---|---|
| *TRG* S5/PGM 01 | IonXpress_001 | *TRG* S5/PGM 07 | IonXpress_007 | *TRG* S5/PGM 11 | IonXpress_011 |
| *TRG* S5/PGM 02 | IonXpress_002 | *TRG* S5/PGM 08 | IonXpress_008 | *TRG* S5/PGM 12 | IonXpress_012 |
| *TRG* S5/PGM 03 | IonXpress_003 | *TRG* S5/PGM 09 | IonXpress_009 | *TRG* S5/PGM 13 | IonXpress_013 |
| *TRG* S5/PGM 04 | IonXpress_004 | *TRG* S5/PGM 10 | IonXpress_010 | *TRG* S5/PGM 14 | IonXpress_014 |
| Controls | | | | | |
| *TRG* POS (+) Qty. 2 | | | NGS NEG (-) Qty. 2 | | |




**V-J Sequence Frequencies.** The LymphoTrack Dx bioinformatics software provides PDF reports which include Top 10 Merged Read Summary as well as a stacked bar graph depicting the relative frequencies of the V-J rearrangements identified in the sample. To learn more about the LymphoTrack Dx software, please refer to the LymphoTrack Dx Bioinformatics Software section.

## Ordering Information

| Catalog # | Products | Quantity Components |
|---|---|---|
| 9-227-0019 | LymphoTrack® Dx *TRG* Assay Kit A - MiSeq® | 8 indices - 5 sequencing reactions each |
| 9-227-0009 | LymphoTrack® Dx *TRG* Assay Panel - MiSeq® | 24 indices - 5 sequencing reactions each |
| 9-500-0009 | LymphoTrack® Dx Software - MiSeq® | 1 CD complimentary with purchase |
| 9-227-0007 | LymphoTrack® Dx *TRG* Assay - S5/PGM™ | 12 indices - 5 sequencing reactions each |
| 9-500-0007 | LymphoTrack® Dx Software - S5/PGM™ | 1 CD complimentary with purchase |

CE IVD These products are CE-IVD assays for *in vitro* diagnostic use.

# LymphoTrack Dx *TRB* Assay

## Assay Description

The LymphoTrack® Dx *TRB* Assay is an *in vitro* diagnostic product intended for next-generation sequencing (NGS) for the Illumina MiSeq® instrument. The assay will determine the frequency distribution of *TRB* Vβ-Jβ gene rearrangements in patients suspected of having lymphoproliferative disease. This assay aids in the identification of lymphoproliferative disorders.

## Summary and Explanation of the Test

In contrast to the IdentiClone® fragment analysis assays for clonality that utilizes three master mixes, this NGS-based assay contains a single multiplex master mix to target conserved V and J regions of the *TRB* gene locus described in lymphoid malignancies, thereby, reducing sample DNA requirements and simplifying the testing workflow. The LymphoTrack Dx *TRB* master mix primers are designed with Illumina® adapters and 8 (Kit A) or 24 (Panel) different indices. This allows amplicons generated from different indexed *TRB* master mixes to be pooled into a single library for loading onto one MiSeq® flow cell.

The associated LymphoTrack Dx Software is capable of sorting complex NGS data by gene target, offering a second layer of multiplexing. This provides users the ability to reduce per sample testing costs by sequencing amplicons from any LymphoTrack Dx MiSeq® Assay (e.g. *TRB*, *TRG*, *IGH*, *IGK*) at the same time. In addition, the LymphoTrack Dx Software provides an easy and streamlined method for visualization of data. Guidelines to interpret samples for evidence or no evidence of clonality are included in the instructions for use provided with each kit.

Positive clonal and negative polyclonal DNA controls are included in kits.

## Background

The LymphoTrack Dx *TRB* Assays represent a significant improvement over fragment analysis methods for clonality testing by providing two important and complementary uses:

1. Detection of initial clonal populations.
2. Identification of sequence information required to track clonal rearrangements in subsequent samples.

Analysis of the rearranged *TRB* locus increases the probability of identifying T cell receptor gene rearrangements, as compared to testing for *TRG* gene rearrangements only. As a result, combining the analysis of *TRB* and *TRG* loci increases the sensitivity of clonality detection.

The human T-cell receptor beta (*TRB*) gene locus on chromosome 7 (7q34) includes 65 Vβ (variable) gene segments, followed by two separate clusters of genes each containing a Dβ (diversity) gene, several Jβ (joining) genes, and a Cβ (constant) region spread over 685 kilobases. The 2 Cβ genes, *TRBC1* and *TRBC2*, encode highly homologous products with no functional difference.

During lymphoid cell development, antigen receptor genes undergo somatic gene rearrangements. Specifically, during T-cell development genes encoding *TRB* molecules are assembled from multiple polymorphic gene segments that generate Vβ - Dβ - Jβ combinations unique in both length and sequence.

Since leukemias and lymphomas originate from the malignant transformation of individual lymphoid cells, all leukemias and lymphomas generally share one or more cell-specific or "clonal" antigen receptor gene rearrangements. Therefore, tests that detect *TRB* clonal rearrangements can be useful in the study of B- and T-cell malignancies.

## Specimen Requirement

50 ng of high-quality genomic DNA.

## References

1. EP Rock et al., J *Exp Med* 179 (1): 323-8 (1994).
2. JE Miller et al., *Molecular Genetic Pathology* (2nd ed.). Springer

## Simplified Representation of the *TRB* Gene


Depicted are the variable (Vβ), diversity (Dβ), and joining (Jβ) gene regions involved in *TRB* gene rearrangements, in addition to the downstream consensus (Cβ) gene regions.

# LymphoTrack® Dx

*TRB* Assay



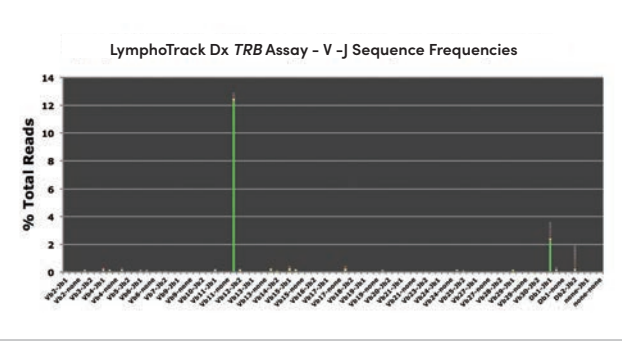
## Reagents - MiSeq® Detection

### Kit A Components

| Master Mix Name | Index # |
|---|---|
| *TRB* MiSeq 01 | A001 |
| *TRB* MiSeq 02 | A002 |
| *TRB* MiSeq 03 | A003 |
| *TRB* MiSeq 04 | A004 |
| *TRB* MiSeq 05 | A005 |
| *TRB* MiSeq 06 | A006 |
| *TRB* MiSeq 07 | A007 |
| *TRB* MiSeq 08 | A008 |
| Controls | |
| *TRB* POS (+) Qty. 1 | NGS NEG (-) Qty. 1 |

### Panel Components (includes all master mixes from Kit A plus the items below)

| Master Mix Name | Index # | Master Mix Name | Index # |
|---|---|---|---|
| *TRB* MiSeq 09 | A009 | *TRB* MiSeq 18 | A018 |
| *TRB* MiSeq 10 | A010 | *TRB* MiSeq 19 | A019 |
| *TRB* MiSeq 11 | A011 | *TRB* MiSeq 20 | A020 |
| *TRB* MiSeq 12 | A012 | *TRB* MiSeq 21 | A021 |
| *TRB* MiSeq 13 | A013 | *TRB* MiSeq 22 | A022 |
| *TRB* MiSeq 14 | A014 | *TRB* MiSeq 23 | A023 |
| *TRB* MiSeq 15 | A015 | *TRB* MiSeq 25 | A025 |
| *TRB* MiSeq 16 | A016 | *TRB* MiSeq 27 | A027 |
| Controls | | | |
| *TRB* POS (+) Qty. 3 | | NGS NEG (-) Qty. 3 | |




**V-J Sequence Frequencies.** The LymphoTrack Dx bioinformatics software provides a stacked bar graph depicting the relative frequencies for the 200 most prevalent rearrangements sequenced and identified in the sample. To learn more about the LymphoTrack Dx software, please refer to the LymphoTrack Dx Bioinformatics Software section.

## Ordering Information

| Catalog # | Products | Quantity Components |
|---|---|---|
| 9-225-0009 | LymphoTrack® Dx *TRB* Assay Kit A - MiSeq® | 8 indices - 5 sequencing reactions each |
| 9-225-0019 | LymphoTrack® Dx *TRB* Assay Panel - MiSeq® | 24 indices - 5 sequencing reactions each |
| 9-500-0009 | LymphoTrack® Dx Software - MiSeq® | 1 CD complimentary with purchase |

CE IVD These products are CE-IVD assays for *in vitro* diagnostic use.

# LymphoTrack Dx Bioinformatics Software

## Software Use

The LymphoTrack Dx Bioinformatics Software package is provided with each LymphoTrack Dx Assay to analyze raw FASTQ files for clonality analysis of single or multiple target data sets (*IGHV* Leader, *IGH* FR1, *IGH* FR2, *IGH* FR3, *IGK, TRB*, or *TRG*). For data generated with LymphoTrack Dx *IGHV* Leader or *IGH* FR1 Assays, the software provides additional information, including the rate of somatic hypermutation and whether a clone would be functional based upon the presence of a premature stop codon. The software can also predict whether an open reading frame would be in- or out-of-frame.

The provided software is composed of two distinct parts:

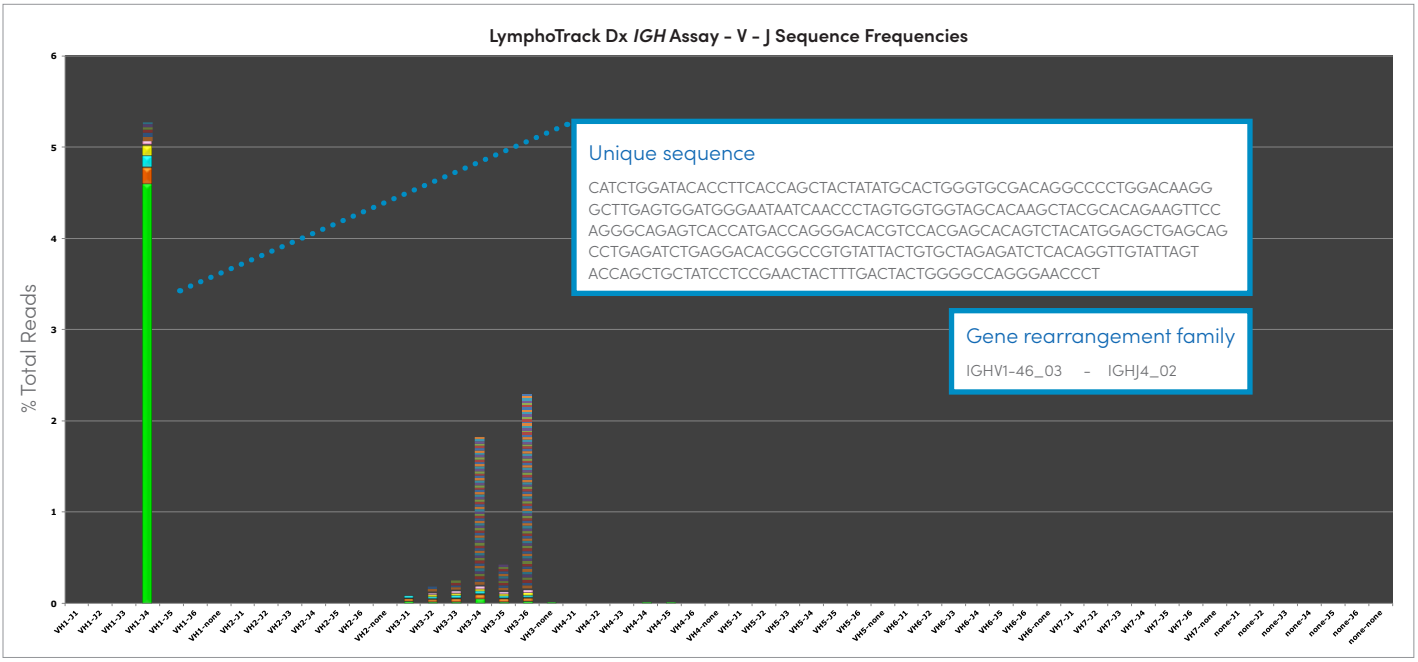
1. A bioinformatics Data Analysis Application
2. Microsoft Excel® Data Visualization spreadsheets and automated Sample-to-PDF Reports for streamlined data analysis for VJ usage and VJ sequence frequency graphs.

## Minimum Software Requirements

- Processor: Intel Core 2 Duo or newer CPU recommended.
- Hard Drive: At least 80 GB of free disk space is required; 250 GB recommended.
- RAM: 4 GB required; 8 GB or more recommended.
- Operating System: Windows 7 (64-bit) is required.
- Java 8 for 64-bit operating systems or higher (Java configured for 32-bit operating systems is not compatible with the LymphoTrack Dx Software – MiSeq or LymphoTrack Dx Software - S5/PGM). The most recent version of Java can be downloaded directly from Oracle at http://www.java.com/.
- A PDF reader, such as Adobe Acrobat Reader, to visualize data reports generated by the LymphoTrack Reporter.
- Microsoft Office Excel 2007, 2010, or 2013 with Macro support enabled is required for data visualization.
- A CD-ROM drive.
- The LymphoTrack Dx Software - MiSeq has been validated for use with Windows 7 configured for English (United States) language and default display size settings, and Microsoft Excel 2007/2010/2013 for alternate data visualization. Use of other Windows/Excel versions and/or language/display size settings may not be compatible.
- The LymphoTrack Dx Software - S5/PGM has been validated for use with Microsoft Excel 2007/2010/2013/2016 and Windows 7 or Windows 10 configured for English (United States) language and default display size settings, and for alternate data visualization. Use of other Windows/Excel versions and/or language/display size settings may not be compatible.

* If a CD-ROM drive is not available, please contact us at: support@invivoscribe.com

## Sequence Frequency Graph




The stacked bar graph depicts the top 200 sequencing reads for a sample. Each individual colored bar represents a unique population of cells. Different colors stacked at the same point on the x-axis represent unique sequences that utilize the same V and J gene families. The amplicons of these products vary in sequence and may also vary in product size.

## Sequencing Summary

Using the merged read summary, along with the easy to follow flow charts in the accompanying LymphoTrack Dx Assay instructions for use (IFU), interpretation is quick and easy.

| (1) Sample Name | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| (1) Total reads = 32,458 | | | | | | | | | | | |
| | (2) | | | (3) | | (4) | | (5) | | | |
| Rank | Sequence | Length | Merge count | V-gene | J-gene | % Total reads | Cumulative % | Mutation rate partial V-gene (%) | In-frame (Y/N) | No stop codon (Y/N) | V-coverage |
| 1 | TTCTCGTGGTG | 455 | 29603 | IGHV4-59_08 | IGHJ4_02 | 9.93 | 9.93 | 11.26 | Y | Y | 98.63 |
| 2 | CTCGCCCTCCT | 463 | 205 | IGHV5-51_01 | IGHJ4_02 | 0.07 | 9.99 | 0.00 | Y | Y | 99.66 |
| 3 | GGTTTTCCTTG | 484 | 201 | IGHV3-7_01 | IGHJ4_02 | 0.07 | 10.06 | 7.77 | Y | Y | 100.00 |
| 4 | CTCGCCCTCCT | 463 | 185 | IGHV5-51_01 | IGHJ5_02 | 0.06 | 10.12 | 6.08 | Y | Y | 99.32 |
| 5 | CTCGCCCTCCT | 469 | 170 | IGHV5-51_01 | IGHJ4_02 | 0.06 | 10.18 | 0.00 | Y | Y | 99.32 |
| 6 | CTCGCCCTCCT | 466 | 160 | IGHV5-51_01 | IGHJ4_02 | 0.05 | 10.23 | 0.00 | Y | Y | 99.66 |
| 7 | CTGCTGCTGAC | 460 | 159 | IGHV2-5_10 | IGHJ5_02 | 0.05 | 10.29 | 8.08 | Y | Y | 97.64 |
| 8 | GGTTTTCCTTG | 493 | 156 | IGHV3-48_02 | IGHJ6_02 | 0.05 | 10.34 | 3.72 | Y | Y | 98.99 |
| 9 | CTCGCCCTCCT | 334 | 153 | IGHV5-51_02 | IGHJ2_01 | 0.05 | 10.39 | 3.72 | Y | N | 27.70 |
| 10 | CTCGCCCTCCT | 334 | 152 | IGHV5-51_02 | IGHJ2_01 | 0.05 | 10.44 | 3.38 | Y | N | 26.01 |

(1) The sample name is clearly identified and the total number of reads (= Read Depth) generated for the sample is provided. Following the IFU, it is easy to determine whether the data generated for a sample can be assessed for the presence or absence of clonality.

(2) The sequence of clonal populations is provided and populations are ranked from most abundant to least prevalent. Sequences that differ by 1-2 basepairs are automatically merged to account for possible sequencing errors and to improve the accuracy and ease of sample interpretation.

(3) Sequences are aligned with reference genes to allow for easy identification of specific types of gene rearrangements such as *IGHV*3-21, which is characteristic of some CLL cases and correlates with a poor prognosis.

(4) The percentage that a unique sequence contributes to the total number of reads for a sample is calculated. Following the guidelines in the IFU, samples can be interpreted for the evidence indicating the presence or absence of clonality.

(5) For the LymphoTrack Dx *IGHV* Leader and *IGH* FR1 Assays, the somatic hypermutation status of a sequence is automatically calculated by comparing the identified sequence with a germline reference. In addition, predictions on whether the sequence would be functional can be drawn by the provided information regarding the presence of a premature stop codon or an open reading frame that is out-of-frame.

## Ordering Information

| Catalog # | Products | Quantity Components |
|---|---|---|
| 9-500-0009 | LymphoTrack® Dx Software - MiSeq® | 1 CD complimentary with kit purchase |
| 9-500-0007 | LymphoTrack® Dx Software - S5/PGM™ | 1 CD complimentary with kit purchase |

CE IVD These products are CE-IVD assays for *in vitro* diagnostic use.

# LymphoTrack® Assays

## Next-Generation Sequencing (NGS) RUO Assays

## Key Benefits

- One-step PCR for amplicon and library generation
- Identify and assess mutation status of B- and T-cell gene rearrangements
- Sequence amplicons from any LymphoTrack kit together
- Included bioinformatics software for easy analysis and interpretation
- Same reagents for clonality, somatic hypermutation (SHM), minimal residual disease (MRD) testing, and tracking/monitoring of immunotherapy constructs

LymphoTrack®
Assays

# LymphoTrack Assay kits are designed for the identification of gene rearrangements in hematologic samples utilizing next-generation sequencing (NGS) technologies.

These assays take advantage of the wealth and depth of NGS data generated with either the Illumina® MiSeq® or the Thermo Fisher Scientific® Ion PGM™ and Ion S5™ platforms. They offer significant improvements over conventional clonality testing methods, by providing the distribution of gene rearrangements, DNA sequences, the mutational status (*IGH* only), and the ability to track specific gene rearrangements all with the same workflow.

Primers included in the master mixes are designed with Illumina® adapters and indices (8 or 24 indices configurations for a total possible 24 or *48 unique indices [*FR1 only] per framework kits) or Thermo Fisher adapters and indices (12 indices per framework kits). By offering multiple kit configurations (8-or-24 indices for MiSeq®, 12 for Ion S5/PGM™), Invivoscribe further provides laboratories the ability to choose the optimal kit for their sample throughput and read-depth requirements. Testing costs can be reduced by sequencing in a single run, with the possibility to combine: a) samples with up to 48 different indices and b) amplicons from other LymphoTrack Assays.

Research Use Only (RUO) assays are not for sale in Europe and other global markets where equivalent CE-IVD assays are available and registered with the appropriate regulatory agencies. For more information regarding the CE-marked LymphoTrack® Dx Assays, please refer to the preceding pages.

# LymphoTrack *IGHV* Leader Somatic Hypermutation Assay

## Assay Uses

This research use only (RUO) assay for next-generation sequencing (NGS), identifies clonal *IGH* VH-JH rearrangements, the associated VH-JH DNA sequences, and the frequency distribution of VH region and JH region segment utilization. The assay also uses the Illumina® MiSeq® platform to define the extent of somatic hypermutation (SHM) present in the *IGHV* gene of analyzed samples. If you would like to test for *IGHV* somatic hypermutation using the Thermo Fisher® Ion PGM™ or Ion S5™ platform please refer to the LymphoTrack *IGH* FR1 Assay (7-121-0007).

## Summary and Explanation of the Test

The LymphoTrack *IGHV* Leader Somatic Hypermutation Assay for NGS provides significant improvements over clonality assays using fragment analysis and Sanger sequencing. The assay efficiently detects the majority of *IGH* gene rearrangements using a single multiplex master mix, identifies the DNA sequence specific for each clonal gene rearrangement, and calculates the degree of SHM for each sample.

The master mixes included in this assay target the Leader (VHL) and the joining (JH) gene regions of *IGH* and are designed with Illumina® adapters and indices (8 included in Kit A and 24 included in Panels). Including adapters and indices in the primer design allows for a one-step PCR approach to generate sequence-ready amplicons, followed by direct pooling of samples for sequencing on a Illumina® MiSeq® flow cell.

The included LymphoTrack bioinformatics software enables simplified analysis and visualization of data generated from this assay.

Positive (clonal positive, SHM negative), negative (polyclonal), and SHM positive (clonal positive, SHM positive) controls are included in the kit. Primers included in the master mixes are designed with Illumina® adapters and indices (8 or 24 indices per framework kits). This allows for a one-step PCR reaction to generate sequence-ready amplicons and pooling of several different samples on the same Illumina® MiSeq® flow cell. The LymphoTrack bioinformatics software enables easy analysis and visualization of data and the LymphoTrack MRD Software allows sequences to be tracked in subsequent samples. Please see the LymphoTrack MRD software section to learn how the LymphoTrack Assays can be applied to MRD studies, or email marketing@invivoscribe.com.

## Background

The human immunoglobulin heavy chain (*IGH*) gene locus on chromosome 14 (14q32.3) includes 46 - 52 functional and 30 nonfunctional variable (VH) gene segments, 27 functional diversity (DH) gene segments, and 6 functional joining (JH) gene segments spread over 1250 kilobases.

During B-cell development, genes encoding the *IGH* molecules are assembled from multiple polymorphic gene segments that undergo rearrangements and selection, generating VH-DH-JH combinations that are unique in both length and sequence for each cell.

Since leukemias and lymphomas originate from the malignant transformation of individual lymphoid cells, all leukemias and lymphomas generally share one or more cell-specific or "clonal" antigen receptor gene rearrangements. Therefore, tests that detect *IGH* clonal rearrangements can be useful in the study of B-cell malignancies. An additional level of diversity is generated in the antigen receptors by somatic point mutations in the variable regions and this mutation status provides important prognostic information for chronic lymphocytic leukemia (CLL) and small lymphocytic lymphoma (SLL). In addition, NGS methods can improve disease stratification and elucidate subclone gene profiles.

## Specimen Requirement

50 ng of high-quality genomic DNA.

## References

1. B Stamatopoulous et al., *Leukemia* 4:837-845 (2017).
2. F Davi et al., *Leukemia* 22: 212-214 (2008).
3. JE Miller et al., *Molecular Genetic Pathology* (2nd ed.). Springer Science & Business Media. 2013: 302.2.7.13 and 30.2.7.18.
4. KJ Trainor et al., *Blood* 75: 2220-2222 (1990).
5. P Ghia et al., *Leukemia* 21: 2-3 (2007).
6. P Ghia et al., *Blood* 105: 1678-685 (2005).
7. S Tonegawa. *Nature* 302: 575-581 (1983).


Simple representation of the organization of the immunoglobulin heavy chain (*IGH*) gene on chromosome 14. Depicted are the variable region (VH) genes and downstream consensus joining region genes (JH) that are involved in rearrangements. Upstream of the variable gene segments, the leader sequence (VHL) is also depicted. Diversity region genes are not depicted.

## Reagents - MiSeq® Detection

### Kit A Components

| Master Mix Name | Index # |
|---|---|
| *IGH* Leader MiSeq 01 | A001 |
| *IGH* Leader MiSeq 02 | A002 |
| *IGH* Leader MiSeq 03 | A003 |
| *IGH* Leader MiSeq 04 | A004 |
| *IGH* Leader MiSeq 05 | A005 |
| *IGH* Leader MiSeq 06 | A006 |
| *IGH* Leader MiSeq 07 | A007 |
| *IGH* Leader MiSeq 08 | A008 |
| **Controls** | |
| *IGH* SHM POS (+) Qty. 1 | |
| *IGH* POS (+) Qty. 1 | |
| NGS NEG (-) Qty. 1 | |

### Panel Components (includes all master mixes from Kit A plus the items below)

| Master Mix Name | Index # | Master Mix Name | Index # |
|---|---|---|---|
| *IGH* Leader MiSeq 09 | A009 | *IGH* Leader MiSeq 18 | A018 |
| *IGH* Leader MiSeq 10 | A010 | *IGH* Leader MiSeq 19 | A019 |
| *IGH* Leader MiSeq 11 | A011 | *IGH* Leader MiSeq 20 | A020 |
| *IGH* Leader MiSeq 12 | A012 | *IGH* Leader MiSeq 21 | A021 |
| *IGH* Leader MiSeq 13 | A013 | *IGH* Leader MiSeq 22 | A022 |
| *IGH* Leader MiSeq 14 | A014 | *IGH* Leader MiSeq 23 | A023 |
| *IGH* Leader MiSeq 15 | A015 | *IGH* Leader MiSeq 25 | A025 |
| *IGH* Leader MiSeq 16 | A016 | *IGH* Leader MiSeq 27 | A027 |
| **Controls** | | | |
| *IGH* SHM POS (+) Qty. 3 | | | |
| *IGH* POS (+) Qty. 3 | | | |
| NGS NEG (-) Qty. 3 | | | |

| Rank | Sequence | Length | Merge count | V-gene | J-gene | % Total reads | Cumulative % | Mutation rate partial V-gene (%) | In-frame (Y/N) | No stop codon (Y/N) | V-coverage |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | TTCTCGTGGTG | 455 | 29603 | IGHV4-59_08 | IGHJ4_02 | 9.93 | 9.93 | 11.26 | Y | Y | 98.63 |
| 2 | CTCGCCCTCCT | 463 | 205 | IGHV5-51_01 | IGHJ4_02 | 0.07 | 9.99 | 0.00 | Y | Y | 99.66 |
| 3 | GGTTTTCCTTG | 484 | 201 | IGHV3-7_01 | IGHJ4_02 | 0.07 | 10.06 | 7.77 | Y | Y | 100.00 |
| 4 | CTCGCCCTCCT | 463 | 185 | IGHV5-51_01 | IGHJ5_02 | 0.06 | 10.12 | 6.08 | Y | Y | 99.32 |
| 5 | CTCGCCCTCCT | 469 | 170 | IGHV5-51_01 | IGHJ4_02 | 0.06 | 10.18 | 0.00 | Y | Y | 99.32 |
| 6 | CTCGCCCTCCT | 466 | 160 | IGHV5-51_01 | IGHJ4_02 | 0.05 | 10.23 | 0.00 | Y | Y | 99.66 |
| 7 | CTGCTGCTGAC | 460 | 159 | IGHV2-5_10 | IGHJ5_02 | 0.05 | 10.29 | 8.08 | Y | Y | 97.64 |
| 8 | GGTTTTCCTTG | 493 | 156 | IGHV3-48_02 | IGHJ6_02 | 0.05 | 10.34 | 3.72 | Y | Y | 98.99 |
| 9 | CTCGCCCTCCT | 334 | 153 | IGHV5-51_02 | IGHJ2_01 | 0.05 | 10.39 | 3.72 | Y | N | 27.70 |
| 10 | CTCGCCCTCCT | 334 | 152 | IGHV5-51_02 | IGHJ2_01 | 0.05 | 10.44 | 3.38 | Y | N | 26.01 |

Example Data. Depicted are the top 10 sequences from a read summary generated by the LymphoTrack Software - MiSeq®. Highlighted columns represent fields that are unique to SHM analysis and include the SHM mutation rate and predictions pertaining to whether a sequence is in-frame or contains a premature stop codon. To learn more about the LymphoTrack software, please refer to the LymphoTrack Bioinformatics Software section.

## Ordering Information

| Catalog # | Products | Quantity Components |
|---|---|---|
| 7-121-0059 | LymphoTrack® *IGHV* Leader Somatic Hypermutation Assay Kit A - MiSeq® | 8 indices - 5 sequencing reactions each |
| 7-121-0069 | LymphoTrack® *IGHV* Leader Somatic Hypermutation Assay Panel - MiSeq® | 24 indices - 5 sequencing reactions each |
| 7-500-0009 | LymphoTrack® Software - MiSeq® | 1 CD complimentary with purchase |

# LymphoTrack *IGH* FR1/FR2/FR3 Assays

## Assay Uses

These research use only (RUO) assays for next-generation sequencing (NGS), identify clonal *IGH* VH-JH rearrangements, the associated VH-JH region DNA sequences, the frequency distribution of VH region and JH region segment utilization. The LymphoTrack FR1 can also identify the degree of somatic hypermutation (SHM) of rearranged genes using the Illumina® MiSeq®, Thermo Fisher Scientific® Ion PGM™ or Ion S5™. The LymphoTrack *IGH* FR1, *IGH* FR2, and *IGH* FR3 Assays contain primers that target the conserved framework 1 (FR1), framework 2 (FR2), and framework 3 (FR3) regions, respectively. The LymphoTrack *IGH* FR1/2/3 Assay kits contain the master mixes of all three frameworks.

## Summary and Explanation of the Test

The LymphoTrack *IGH* Assays represent a significant improvement over clonality assays that utilize fragment analysis by providing four important and complementary uses:

1. Detection of clonal populations.
2. Identification of sequence information and VH-JH segment utilization.
3. The LymphoTrack *IGH* FR1 Assays provide the degree of SHM of the immunoglobulin variable heavy chain (*IGHV*).
4. The ability to track sequences in subsequent samples with the Invivoscribe LymphoTrack MRD Software.

Primers included in the master mixes are designed with Illumina® adapters and indices (8 included in Kit A, 24 included in the Panel, and an independent 24 included in the Panel B) or Thermo Fisher adaptors and indices (12 indices per framework kits). This allows for a one-step PCR reaction to generate sequence-ready amplicons and pooling of several different samples on the same Illumina® MiSeq® cell, Ion S5 or Ion PGM chip. The LymphoTrack bioinformatics software enables easy analysis and visualization of data and the LymphoTrack MRD Software allows sequences to be tracked in subsequent samples. Please see the LymphoTrack MRD software section to learn how the LymphoTrack Assays can be applied to MRD studies, or email marketing@invivoscribe.com.

Positive clonal (SHM negative) and negative polyclonal DNA controls are included in kits. A clonal SHM positive control can be purchased separately (4-088-0008).

## Background

The human immunoglobulin heavy chain (*IGH*) gene locus on chromosome 14 (14q32.3) includes 46-52 functional and 30 nonfunctional variable (VH) gene segments, 27 functional diversity (DH) gene segments, and 6 functional joining (JH) gene segments spread over 1250 kilobases.

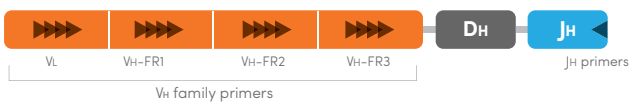
During development of lymphoid cells, the antigen receptor genes go through somatic gene rearrangements. For example, during B-cell development, genes encoding the IGH molecules are assembled from multiple polymorphic gene segments that undergo rearrangements and selection, generating VH-DH-JH combinations. Since leukemias and lymphomas originate from the malignant transformation of individual lymphoid cells, all leukemias and lymphomas generally share one or more cell-specific or "clonal" antigen receptor gene rearrangements. Therefore, tests that detect *IGH* clonal populations can be useful in the study of B- and T-cell malignancies.

## Specimen Requirement

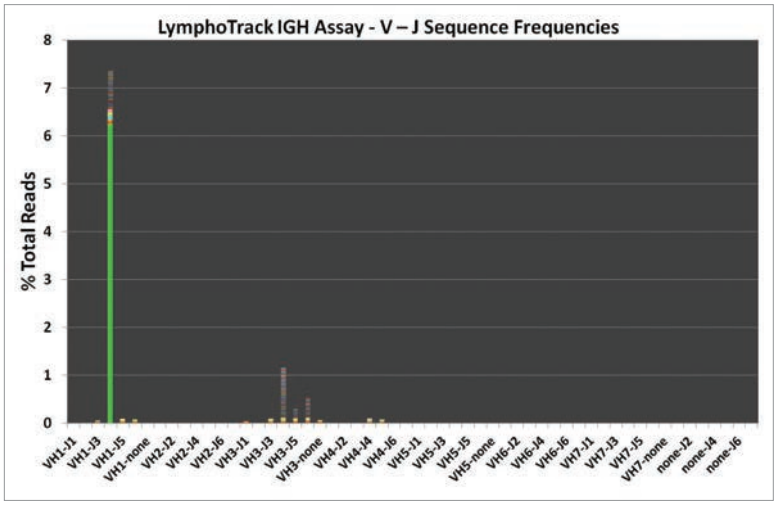
50 ng of high-quality genomic DNA.

## References

1. S Tonegawa. *Nature* 302: 575-581 (1983).
2. JE Miller et al., *Molecular Genetic Pathology* (2nd ed.). Springer Science & Business Media. 2013: 30.2.7.13 and 30.2.7.18.
3. KJ Trainor et al., *Blood* 75: 2220-2222 (1990).
4. P Ghia. *Leukemia* 21: 1-3 (2007).




Simple representation of the organization of the immunoglobulin heavy chain (*IGH*) gene locus on chromosome 14. Depicted are the variable region (VH) genes and downstream consensus joining region genes segments (JH) that are involved in rearrangements.




**V-J Sequence Frequencies.** The LymphoTrack Software provides a stacked bar graph depicting the relative frequencies for the 200 most prevalent VH-JH rearrangements identified in a sample. To learn more about the LymphoTrack software, please refer to the LymphoTrack Bioinformatics Software section.

## Ordering Information

| Catalog # | Products | Quantity Components |
|---|---|---|
| 7-121-0129 | LymphoTrack® *IGH* FR1/2/3 Assay Kit A - MiSeq® | Indices 1-8 (5 sequencing reactions each) |
| 7-121-0139 | LymphoTrack® *IGH* FR1/2/3 Assay Panel - MiSeq® | Indices 1-24 (5 sequencing reactions each) |
| 7-121-0009 | LymphoTrack® *IGH* FR1 Assay Kit A - MiSeq® | Indices 1-8 (5 sequencing reactions each) |
| 7-121-0039 | LymphoTrack® *IGH* FR1 Assay Panel - MiSeq® | Indices 1-24 (5 sequencing reactions each) |
| 7-121-0149 | LymphoTrack® *IGH* FR1 Assay Panel B - MiSeq® **NEW!** | Indices 25-48 (5 sequencing reaction each) |
| 7-121-0089 | LymphoTrack® *IGH* FR2 Assay Kit A - MiSeq® | Indices 1-8 (5 sequencing reactions each) |
| 7-121-0099 | LymphoTrack® *IGH* FR2 Assay Panel - MiSeq® | Indices 1-24 (5 sequencing reactions each) |
| 7-121-0109 | LymphoTrack® *IGH* FR3 Assay Kit A - MiSeq® | Indices 1-8 (5 sequencing reactions each) |
| 7-121-0119 | LymphoTrack® *IGH* FR3 Assay Panel - MiSeq® | Indices 1-24 (5 sequencing reactions each) |
| 7-500-0009 | LymphoTrack® Software - MiSeq® | 1 CD complimentary with purchase |
| 7-121-0057 | LymphoTrack® *IGH* FR1/2/3 Assay - S5/PGM™ | 12 + 12 + 12 indices - 5 sequencing reactions each |
| 7-121-0007 | LymphoTrack® *IGH* FR1 Assay - S5/PGM™ | 12 indices - 5 sequencing reactions each |
| 7-121-0037 | LymphoTrack® *IGH* FR2 Assay - S5/PGM™ | 12 indices - 5 sequencing reactions each |
| 7-121-0047 | LymphoTrack® *IGH* FR3 Assay - S5/PGM™ | 12 indices - 5 sequencing reactions each |
| 7-500-0007 | LymphoTrack® Software - S5/PGM™ | 1 CD complimentary with purchase |
| 7-500-0008 | LymphoTrack® MRD Software | 1 CD complimentary with purchase |

# LymphoTrack *IGH* FR1/FR2/FR3 Assays continued

## Reagents - MiSeq® Detection

The LymphoTrack *IGH* FR1/2/3 Assays contain components from respective individual FR Kit A's or Panels.

| LymphoTrack *IGH* FR1 Components | | | LymphoTrack *IGH* FR2 Components | | | LymphoTrack *IGH* FR3 Components | | |
|---|---|---|---|---|---|---|---|---|
| Master Mix Name | Index # | | Master Mix Name | Index # | | Master Mix Name | Index # | |
| *IGH* FR1 MiSeq 01 | A001 | KIT A | *IGH* FR2 MiSeq 01 | A001 | KIT A | *IGH* FR3 MiSeq 01 | A001 | KIT A |
| *IGH* FR1 MiSeq 02 | A002 | | *IGH* FR2 MiSeq 02 | A002 | | *IGH* FR3 MiSeq 02 | A002 | |
| *IGH* FR1 MiSeq 03 | A003 | | *IGH* FR2 MiSeq 03 | A003 | | *IGH* FR3 MiSeq 03 | A003 | |
| *IGH* FR1 MiSeq 04 | A004 | | *IGH* FR2 MiSeq 04 | A004 | | *IGH* FR3 MiSeq 04 | A004 | |
| *IGH* FR1 MiSeq 05 | A005 | | *IGH* FR2 MiSeq 05 | A005 | | *IGH* FR3 MiSeq 05 | A005 | |
| *IGH* FR1 MiSeq 06 | A006 | | *IGH* FR2 MiSeq 06 | A006 | | *IGH* FR3 MiSeq 06 | A006 | |
| *IGH* FR1 MiSeq 07 | A007 | | *IGH* FR2 MiSeq 07 | A007 | | *IGH* FR3 MiSeq 07 | A007 | |
| *IGH* FR1 MiSeq 08 | A008 | | *IGH* FR2 MiSeq 08 | A008 | | *IGH* FR3 MiSeq 08 | A008 | |
| *IGH* FR1 MiSeq 09 | A009 | PANEL | *IGH* FR2 MiSeq 09 | A009 | PANEL | *IGH* FR3 MiSeq 09 | A009 | PANEL |
| *IGH* FR1 MiSeq 10 | A010 | | *IGH* FR2 MiSeq 10 | A010 | | *IGH* FR3 MiSeq 10 | A010 | |
| *IGH* FR1 MiSeq 11 | A011 | | *IGH* FR2 MiSeq 11 | A011 | | *IGH* FR3 MiSeq 11 | A011 | |
| *IGH* FR1 MiSeq 12 | A012 | | *IGH* FR2 MiSeq 12 | A012 | | *IGH* FR3 MiSeq 12 | A012 | |
| *IGH* FR1 MiSeq 13 | A013 | | *IGH* FR2 MiSeq 13 | A013 | | *IGH* FR3 MiSeq 13 | A013 | |
| *IGH* FR1 MiSeq 14 | A014 | | *IGH* FR2 MiSeq 14 | A014 | | *IGH* FR3 MiSeq 14 | A014 | |
| *IGH* FR1 MiSeq 15 | A015 | | *IGH* FR2 MiSeq 15 | A015 | | *IGH* FR3 MiSeq 15 | A015 | |
| *IGH* FR1 MiSeq 16 | A016 | | *IGH* FR2 MiSeq 16 | A016 | | *IGH* FR3 MiSeq 16 | A016 | |
| *IGH* FR1 MiSeq 18 | A018 | | *IGH* FR2 MiSeq 18 | A018 | | *IGH* FR3 MiSeq 18 | A018 | |
| *IGH* FR1 MiSeq 19 | A019 | | *IGH* FR2 MiSeq 19 | A019 | | *IGH* FR3 MiSeq 19 | A019 | |
| *IGH* FR1 MiSeq 20 | A020 | | *IGH* FR2 MiSeq 20 | A020 | | *IGH* FR3 MiSeq 20 | A020 | |
| *IGH* FR1 MiSeq 21 | A021 | | *IGH* FR2 MiSeq 21 | A021 | | *IGH* FR3 MiSeq 21 | A021 | |
| *IGH* FR1 MiSeq 22 | A022 | | *IGH* FR2 MiSeq 22 | A022 | | *IGH* FR3 MiSeq 22 | A022 | |
| *IGH* FR1 MiSeq 23 | A023 | | *IGH* FR2 MiSeq 23 | A023 | | *IGH* FR3 MiSeq 23 | A023 | |
| *IGH* FR1 MiSeq 25 | A025 | | *IGH* FR2 MiSeq 25 | A025 | | *IGH* FR3 MiSeq 25 | A025 | |
| *IGH* FR1 MiSeq 27 | A027 | | *IGH* FR2 MiSeq 27 | A027 | | *IGH* FR3 MiSeq 27 | A027 | |
| *IGH* FR1 MiSeq 17 | A017 | PANEL B | | | | | | |
| *IGH* FR1 MiSeq 24 | A024 | | | | | | | |
| *IGH* FR1 MiSeq 26 | A026 | | | | | | | |
| *IGH* FR1 MiSeq 28 | A028 | | | | | | | |
| *IGH* FR1 MiSeq 29 | A029 | | | | | | | |
| *IGH* FR1 MiSeq 30 | A030 | | | | | | | |
| *IGH* FR1 MiSeq 31 | A031 | | | | | | | |
| *IGH* FR1 MiSeq 32 | A032 | | | | | | | |
| *IGH* FR1 MiSeq 33 | A033 | | | | | | | |
| *IGH* FR1 MiSeq 34 | A034 | | | | | | | |
| *IGH* FR1 MiSeq 35 | A035 | | | | | | | |
| *IGH* FR1 MiSeq 36 | A036 | | | | | | | |
| *IGH* FR1 MiSeq 37 | A037 | | | | | | | |
| *IGH* FR1 MiSeq 38 | A038 | | | | | | | |
| *IGH* FR1 MiSeq 39 | A039 | | | | | | | |
| *IGH* FR1 MiSeq 40 | A040 | | | | | | | |

## Reagents - MiSeq® Detection cont.

### The LymphoTrack *IGH* FR1/2/3 Assays contain components from respective individual FR Kit A's or Panels.

| LymphoTrack *IGH* FR1 Components | | LymphoTrack *IGH* FR2 Components | | LymphoTrack *IGH* FR3 Components | |
|---|---|---|---|---|---|
| Master Mix Name | Index # | Master Mix Name | Index # | Master Mix Name | Index # |
| *IGH* FR1 MiSeq 41 | A041 | | | | |
| *IGH* FR1 MiSeq 42 | A042 | | | | |
| *IGH* FR1 MiSeq 43 | A043 | | | | |
| *IGH* FR1 MiSeq 44 | A044 | | | | |
| *IGH* FR1 MiSeq 45 | A045 | | | | |
| *IGH* FR1 MiSeq 46 | A046 | | | | |
| *IGH* FR1 MiSeq 47 | A047 | | | | |
| *IGH* FR1 MiSeq 48 | A048 | | | | |
| *IGH* FR1 MiSeq 49 | A049 | | | | |

PANEL B

| Controls in Individual FR (1, 2, or 3) Kit A's | Controls in Individual FR (1,2, or 3) Panels | Controls in Combo FR 1/2/3 Kit A | Controls in Combo FR 1/2/3 Panel |
|---|---|---|---|
| *IGH* POS (+) Qty. 1 | *IGH* POS (+) Qty. 3 | *IGH* POS (+) Qty. 2 | *IGH* POS (+) Qty. 6 |
| NGS NEG (-) Qty. 1 | NGS NEG (-) Qty. 3 | NGS NEG (-) Qty. 2 | NGS NEG (-) Qty. 6 |

**Kit A's contain indices *IGH* FRX A001 to A008. Panels contain all master mixes listed on pg. 40**

## Reagents - Ion S5™/PGM™ Detection

### The LymphoTrack *IGH* FR1/2/3 Assays contain components from respective individual FR Assays.

| LymphoTrack *IGH* FR1 Components | | LymphoTrack *IGH* FR2 Components | | LymphoTrack *IGH* FR3 Components | |
|---|---|---|---|---|---|
| Master Mix Name | Index # | Master Mix Name | Index # | Master Mix Name | Index # |
| *IGH* FR1 S5/PGM 01 | IonXpress_001 | *IGH* FR2 S5/PGM 01 | IonXpress_001 | *IGH* FR3 S5/PGM 01 | IonXpress_001 |
| *IGH* FR1 S5/PGM 02 | IonXpress_002 | *IGH* FR2 S5/PGM 02 | IonXpress_002 | *IGH* FR3 S5/PGM 02 | IonXpress_002 |
| *IGH* FR1 S5/PGM 03 | IonXpress_003 | *IGH* FR2 S5/PGM 03 | IonXpress_003 | *IGH* FR3 S5/PGM 03 | IonXpress_003 |
| *IGH* FR1 S5/PGM 04 | IonXpress_004 | *IGH* FR2 S5/PGM 04 | IonXpress_004 | *IGH* FR3 S5/PGM 04 | IonXpress_004 |
| *IGH* FR1 S5/PGM 07 | IonXpress_007 | *IGH* FR2 S5/PGM 07 | IonXpress_007 | *IGH* FR3 S5/PGM 07 | IonXpress_007 |
| *IGH* FR1 S5/PGM 08 | IonXpress_008 | *IGH* FR2 S5/PGM 08 | IonXpress_008 | *IGH* FR3 S5/PGM 08 | IonXpress_008 |
| *IGH* FR1 S5/PGM 09 | IonXpress_009 | *IGH* FR2 S5/PGM 09 | IonXpress_009 | *IGH* FR3 S5/PGM 09 | IonXpress_009 |
| *IGH* FR1 S5/PGM 10 | IonXpress_010 | *IGH* FR2 S5/PGM 10 | IonXpress_010 | *IGH* FR3 S5/PGM 10 | IonXpress_010 |
| *IGH* FR1 S5/PGM 11 | IonXpress_011 | *IGH* FR2 S5/PGM 11 | IonXpress_011 | *IGH* FR3 S5/PGM 11 | IonXpress_011 |
| *IGH* FR1 S5/PGM 12 | IonXpress_012 | *IGH* FR2 S5/PGM 12 | IonXpress_012 | *IGH* FR3 S5/PGM 12 | IonXpress_012 |
| *IGH* FR1 S5/PGM 13 | IonXpress_013 | *IGH* FR2 S5/PGM 13 | IonXpress_013 | *IGH* FR3 S5/PGM 13 | IonXpress_013 |
| *IGH* FR1 S5/PGM 14 | IonXpress_014 | *IGH* FR2 S5/PGM 14 | IonXpress_014 | *IGH* FR3 S5/PGM 14 | IonXpress_014 |

| Controls in Individual FR (1,2, or 3) Kits | Controls in FR 1/2/3 Kit |
|---|---|
| *IGH* POS (+) Qty. 2 | *IGH* POS (+) Qty. 4 |
| NGS NEG (-) Qty. 2 | NGS NEG (-) Qty. 4 |

# LymphoTrack *IGK* Assay

## Assay Uses

This research use only (RUO) assay for next-generation sequencing (NGS), identifies clonal *IGK* Vκ-Jκ, Vκ-$K_{de}$, and intron-$K_{de}$ (INTR-$K_{de}$) rearrangements, the corresponding DNA sequences, and provides the distribution frequency of Vκ-Jκ, Vκ-$K_{de}$, and INTR-$K_{de}$ segment utilization using the Illumina® MiSeq® or Thermo Fisher Scientific® Ion PGM™ and Ion S5™ platforms.

## Summary and Explanation of the Test

The LymphoTrack *IGK* Assay represents a significant improvement over clonality assays that utilize fragment analysis by providing three important and complementary uses:

1. Detection of clonal populations.
2. Identification of sequence information and gene segment utilization.
3. Ability to track sequences in subsequent samples with the use of the LymphoTrack MRD Software.

Unlike conventional fragment analysis assays, this NGS method utilizes a single multiplex master mix to target conserved regions of *IGK* that are described in lymphoid malignancies. Primers are designed with Illumina® adapters and indices (8-24) or Thermo Fisher Scientific adaptors and indices (12), thereby allowing for a one-step PCR reaction to generate sequence-ready amplicons. To reduce per sample testing costs, amplicons from different samples (amplified with different indexed master mixes) or LymphoTrack kits can be sequenced together on a single Illumina® MiSeq® flow cell, Ion S5™ or PGM™ chips.

The LymphoTrack bioinformatics software enables simplified analysis and visualization of data and the LymphoTrack MRD Software allows identified sequences to be tracked in subsequent samples. Please see the LymphoTrack MRD software section to learn how the LymphoTrack Assays can be applied to MRD studies, or email marketing@invivoscribe.com. Positive (clonal) and negative (polyclonal) DNA controls are included in the kits.

## Background

The human immunoglobulin kappa (*IGK*) gene locus on chromosome 2 (2p11.2) includes 76 V (variable) region genes spanning 7 subgroups and 5 J (joining) region gene segments upstream of the Cκ region. The $K_{de}$, approximately 24 kb downstream of the Jκ-Cκ region, can also rearrange with Vκ gene segments and the isolated recombination signal sequence in the Jκ-Cκ intronic region.

During development of lymphoid cells, antigen receptor genes undergo somatic gene rearrangements. Specifically during B-cell development, genes encoding *IGK* molecules are assembled from multiple polymorphic gene segments that undergo rearrangements generating V-J combinations unique in both length and sequence. Since leukemias and lymphomas originate from the malignant transformation of individual lymphoid cells, all leukemias and lymphomas generally share one or more cell-specific or "clonal" antigen receptor gene rearrangements. Therefore, tests that detect *IGK* clonal populations can be useful in the study of B-cell malignancies and complement *IGH* testing, as the *IGK* receptor is less susceptible to somatic mutations.

## Specimen Requirement

50 ng of high-quality genomic DNA.

## References

1. S Tonegawa et al., *Nature* 302: 575-581 (1983).
2. JE Miller et al., *Molecular Genetic Pathology* (2nd ed.). Springer Science & Business Media. 2013: 30.2.7.13 and 30.2.7.18.
3. KJ Trainor et al., *Blood* 75: 2220-2222 (1990).

## Simplified Representation of the *IGK* Gene


Depicted are the variable region (Vκ) genes or variable intragenic Jκ-Cκ intron (Jκ-Cκ INTR) and downstream consensus joining region genes (Jκ) or kappa deleting element ($K_{de}$) that are involved in *IGK* gene rearrangements.

# Reagents - MiSeq® Detection

## Kit A Components

| Master Mix Name | Index # |
|---|---|
| *IGK* MiSeq 01 | A001 |
| *IGK* MiSeq 02 | A002 |
| *IGK* MiSeq 03 | A003 |
| *IGK* MiSeq 04 | A004 |
| *IGK* MiSeq 05 | A005 |
| *IGK* MiSeq 06 | A006 |
| *IGK* MiSeq 07 | A007 |
| *IGK* MiSeq 08 | A008 |

| Controls | |
|---|---|
| *IGK* POS (+) Qty. 1 | NGS NEG (-) Qty. 1 |

## Panel Components (includes all master mixes from Kit A plus the items below)

| Master Mix Name | Index # | Master Mix Name | Index # |
|---|---|---|---|
| *IGK* MiSeq 09 | A009 | *IGK* MiSeq 18 | A018 |
| *IGK* MiSeq 10 | A010 | *IGK* MiSeq 19 | A019 |
| *IGK* MiSeq 11 | A011 | *IGK* MiSeq 20 | A020 |
| *IGK* MiSeq 12 | A012 | *IGK* MiSeq 21 | A021 |
| *IGK* MiSeq 13 | A013 | *IGK* MiSeq 22 | A022 |
| *IGK* MiSeq 14 | A014 | *IGK* MiSeq 23 | A023 |
| *IGK* MiSeq 15 | A015 | *IGK* MiSeq 25 | A025 |
| *IGK* MiSeq 16 | A016 | *IGK* MiSeq 27 | A027 |

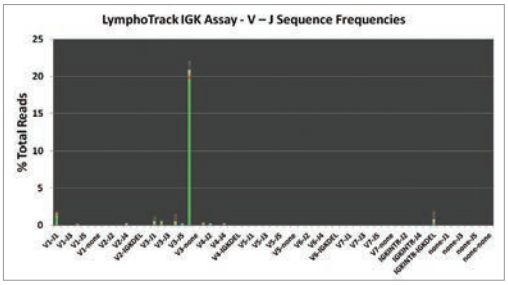
| Controls | |
|---|---|
| *IGK* POS (+) Qty. 3 | NGS NEG (-) Qty. 3 |

# Reagents - Ion S5/PGM™ Detection

## Assay Components

| Master Mix Name | Index # | Master Mix Name | Index # |
|---|---|---|---|
| *IGK* S5/PGM 01 | IonXpress_001 | *IGK* S5/PGM 11 | IonXpress_011 |
| *IGK* S5/PGM 02 | IonXpress_002 | *IGK* S5/PGM 12 | IonXpress_012 |
| *IGK* S5/PGM 04 | IonXpress_004 | *IGK* S5/PGM 13 | IonXpress_013 |
| *IGK* S5/PGM 08 | IonXpress_008 | *IGK* S5/PGM 14 | IonXpress_014 |
| *IGK* S5/PGM 09 | IonXpress_009 | *IGK* S5/PGM 16 | IonXpress_016 |
| *IGK* S5/PGM 010 | IonXpress_010 | *IGK* S5/PGM 17 | IonXpress_017 |

| Controls | |
|---|---|
| *IGK* POS (+) Qty. 2 | NGS NEG (-) Qty. 2 |




**V-J Sequence Frequencies.** The LymphoTrack bioinformatics software provides a stacked bar graph depicting the relative frequencies for the 200 most prevalent rearrangements sequenced and identified in the sample. To learn more about the LymphoTrack software, please refer to the LymphoTrack Bioinformatics Software section.

## Ordering Information

| Catalog # | Products | Quantity Components |
|---|---|---|
| 7-122-0009 | LymphoTrack® *IGK* Assay Kit A - MiSeq® | 8 indices - 5 sequencing reactions each |
| 7-122-0019 | LymphoTrack® *IGK* Assay Panel - MiSeq® | 24 indices - 5 sequencing reactions each |
| 7-500-0009 | LymphoTrack® Software - MiSeq® | 1 CD complimentary with purchase |
| 7-122-0007 | LymphoTrack® *IGK* Assay - S5/PGM™ | 12 indices - 5 sequencing reactions each |
| 7-500-0007 | LymphoTrack® Software - S5/PGM™ | 1 CD complimentary with purchase |
| 7-500-0008 | LymphoTrack® MRD Software* | 1 CD complimentary with purchase |

*MRD Software can be used to track sequences generated by either LymphoTrack Assays - MiSeq® or Ion S5/PGM™.

# LymphoTrack *TRG* Assay

## Assay Uses

This research use only (RUO) assay for next-generation sequencing (NGS) identifies clonal *TRG* V-J rearrangements and the associated V-J region DNA sequences. It also provides the frequency distribution of V-J segment utilization using the Illumina® MiSeq® or Thermo Fisher Scientific® Ion S5™ and PGM™ instruments.

## Summary and Explanation of the Test

The LymphoTrack *TRG* Assay represents a significant improvement over existing clonality assays that utilize fragment analysis by providing three important and complementary uses:

1. Detection of clonal populations.
2. Identification of sequence information and gene segment utilization.
3. Ability to track sequences in subsequent samples with the use of the Invivoscribe MRD Software.

The single multiplex master mix targets all conserved regions within the variable (V) and the joining (J) genes described in lymphoid malignancies. The average PCR amplicon size of the *TRG* gene rearrangements generated using this assay was designed to be compatible with fragmented DNA isolated from challenging samples such as FFPE sections.

Primers are designed with Illumina® adapters and indices (8-24) or Thermo Fisher Scientific adaptors and indices (12), thereby allowing for a one-step PCR reaction to generate sequence-ready amplicons. In addition, amplicons from different samples (amplified with different indexed master mixes) or LymphoTrack kits can be sequenced together on a single Illumina® MiSeq® flow cell Ion S5™ or PGM™ chip to reduce per sample testing costs.

The LymphoTrack bioinformatics software enables simplified analysis and visualization of data generated from this assay and the LymphoTrack MRD Software allows sequences to be tracked in subsequent samples. Please see the LymphoTrack MRD software section to learn how the LymphoTrack Assays can be applied to MRD studies, or email marketing@invivoscribe.com. Positive (clonal) and negative (polyclonal) DNA controls are included in the kits.

## Background

The human T-cell receptor gamma (*TRG*) gene locus on chromosome 7 (7q14) includes 14 variable region (Group I, II, III, and IV), 5 joining region (J) gene segments, and 2 constant (C) genes spread over 200 kilobases.

During development of lymphoid cells, the antigen receptor genes (including gene segments within the *TRG* locus), undergo somatic gene rearrangement. These developmentally regulated gene rearrangements generate V-J combinations that are unique for each cell. Since leukemias and lymphomas originate from the malignant transformation of individual lymphoid cells, all leukemias and lymphomas generally share one or more cell-specific or "clonal" antigen receptor gene rearrangements. Therefore, tests that detect *TRG* clonal populations can be useful in the study of T-cell and certain B-cell malignancies. Since the *TRG* locus rearranges before the *TRB* locus, and all unsuccessful or unproductive rearrangements are retained in the cells, examination of the *TRG* locus can identify both clonal Δ/γ as well as clonal α/β T-cells. Clonal α/β T-cells would be expected to have biallelic *TRG* gene rearrangements.

## Specimen Requirement

50 ng of high-quality genomic DNA.

## References

1. S Tonegawa et al., *Nature* 302: 575-581 (1983).
2. JE Miller et al., *Molecular Genetic Pathology* (2nd ed.). Springer Science & Business Media. 2013: 30.2.7.13 and 30.2.7.18.
3. KJ Trainor et al., *Blood* 75: 2220-2222 (1990).

## Simplified Representation of the *TRG* Gene


Simple representation of the organization of the T-cell receptor gamma gene on chromosome 7. Depicted are the variable region genes (Vγ2–Vγ11) and downstream joining region genes (Jγ1/2–JγP1/2) that are involved in rearrangements in T-cell lymphomas.

# Reagents - MiSeq® Detection

## Kit A Components

| Master Mix Name | Index # |
|---|---|
| *TRG* MiSeq 01 | A001 |
| *TRG* MiSeq 02 | A002 |
| *TRG* MiSeq 03 | A003 |
| *TRG* MiSeq 04 | A004 |
| *TRG* MiSeq 05 | A005 |
| *TRG* MiSeq 06 | A006 |
| *TRG* MiSeq 07 | A007 |
| *TRG* MiSeq 08 | A008 |
| Controls | |
| *TRG* POS (+) Qty. 1 | NGS NEG (-) Qty. 1 |

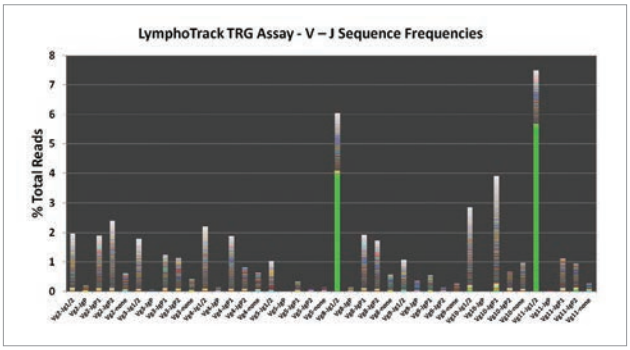
## Panel Components (includes all master mixes from Kit A plus the items below)

| Master Mix Name | Index # | Master Mix Name | Index # |
|---|---|---|---|
| *TRG* MiSeq 09 | A009 | *TRG* MiSeq 18 | A018 |
| *TRG* MiSeq 10 | A010 | *TRG* MiSeq 19 | A019 |
| *TRG* MiSeq 11 | A011 | *TRG* MiSeq 20 | A020 |
| *TRG* MiSeq 12 | A012 | *TRG* MiSeq 21 | A021 |
| *TRG* MiSeq 13 | A013 | *TRG* MiSeq 22 | A022 |
| *TRG* MiSeq 14 | A014 | *TRG* MiSeq 23 | A023 |
| *TRG* MiSeq 15 | A015 | *TRG* MiSeq 25 | A025 |
| *TRG* MiSeq 16 | A016 | *TRG* MiSeq 27 | A027 |
| Controls | | | |
| *TRG* POS (+) Qty. 3 | | NGS NEG (-) Qty. 3 | |

# Reagents - Ion S5/PGM™ Detection

## Assay Components

| Master Mix Name | Index # | Master Mix Name | Index # | Master Mix Name | Index # |
|---|---|---|---|---|---|
| *TRG* S5/PGM 01 | IonXpress_001 | *TRG* S5/PGM 07 | IonXpress_007 | *TRG* S5/PGM 11 | IonXpress_011 |
| *TRG* S5/PGM 02 | IonXpress_002 | *TRG* S5/PGM 08 | IonXpress_008 | *TRG* S5/PGM 12 | IonXpress_012 |
| *TRG* S5/PGM 03 | IonXpress_003 | *TRG* S5/PGM 09 | IonXpress_009 | *TRG* S5/PGM 13 | IonXpress_013 |
| *TRG* S5/PGM 04 | IonXpress_004 | *TRG* S5/PGM 10 | IonXpress_010 | *TRG* S5/PGM 14 | IonXpress_014 |
| Controls | | | | | |
| *TRG* POS (+) Qty. 2 | | | NGS NEG (-) Qty. 2 | | |




**V - J Sequence Frequencies.** The LymphoTrack bioinformatics software provides a stacked bar graph depicting the relative frequencies for the V-J rearrangements identified and sequenced in a sample. To learn more about the LymphoTrack software, please refer to the LymphoTrack Bioinformatics Software section.

## Ordering Information

| Catalog # | Products | Quantity Components |
|---|---|---|
| 7-227-0019 | LymphoTrack® *TRG* Assay Kit A - MiSeq® | 8 indices - 5 sequencing reactions each |
| 7-227-0009 | LymphoTrack® *TRG* Assay Panel - MiSeq® | 24 indices - 5 sequencing reactions each |
| 7-500-0009 | LymphoTrack® Software - MiSeq® | 1 CD complimentary with purchase |
| 7-227-0007 | LymphoTrack® *TRG* Assay - S5/PGM™ | 12 indices - 5 sequencing reactions each |
| 7-500-0007 | LymphoTrack® Software - S5/PGM™ | 1 CD complimentary with purchase |
| 7-500-0008 | LymphoTrack® MRD Software* | 1 CD complimentary with purchase |

*MRD Software can be used to track sequences generated by either LymphoTrack Assays - MiSeq® or Ion S5/PGM™.

# LymphoTrack *TRB* Assay

## Assay Uses

This research use only (RUO) assay for next-generation sequencing (NGS) identifies clonal *TRB* Vβ-(Dβ-)Jβ rearrangements, the associated Vβ-(Dβ-)Jβ region DNA sequences, it provides the frequency distribution of Vβ, Dβ, and Jβ region segment utilization using the Illumina® MiSeq® platform.

## Summary and Explanation of the Test

In contrast to the conventional fragment analysis assays for clonality that utilize three master mixes, this NGS-based assay contains a single multiplex master mix to target conserved V, D, and J regions of the *TRB* gene locus described in lymphoid malignancies. This reduces DNA sample requirements and simplifies the testing workflow. The LymphoTrack *TRB* master mix primers are also designed with Illumina® adapters and 8 indices (Kit A) or 24 indices (Panel). This allows amplicons generated from different indexed *TRB* master mixes to be pooled into a single sequencing library.

The associated LymphoTrack Software is capable of sorting complex NGS data by gene target. This offers a second layer of multiplexing to reduce per sample testing costs by allowing amplicons from any LymphoTrack Assay (*e.g. IGH, IGK, TRB, TRG*) to be sequenced on the same flow cell. In addition, the LymphoTrack Software provides easy visualization of data and the LymphoTrack Minimal Residual Disease Data Analysis Tool allows identified sequences to be tracked and monitored in subsequent samples. Positive clonal and negative polyclonal DNA controls are included in kits. Please see the LymphoTrack MRD software section to learn how the LymphoTrack Assays can be applied to MRD studies, or email marketing@invivoscribe.com.

## Background

The LymphoTrack *TRB* Assay represent a significant improvement over clonality assessment by fragment analysis by providing two important and complementary uses:

1. Detection of initial clonal populations.
2. Identification of sequence information required to track clonal rearrangements in subsequent samples.

Analysis of the rearranged *TRB* locus increases the probability of identifying T-cell receptor gene rearrangements, as compared to testing for *TRG* gene rearrangements only. As a result, combining the analysis of *TRB* and *TRG* loci increases the sensitivity of clonality detection.

The human T-cell receptor beta (*TRB*) gene locus on chromosome 7 (7q34) includes 65 Vβ (variable) gene segments, followed by two separate clusters of genes each containing a Dβ (diversity) gene, several Jβ (joining) genes, and a Cβ (constant) region spread over 685 kilobases. The two Cβ genes, *TRBC1* and *TRBC2*, encode highly homologous products with no functional difference.

During lymphoid cell development, antigen receptor genes undergo somatic gene rearrangements. Specifically, during T-cell development, genes encoding *TRB* molecules are assembled from multiple polymorphic gene segments that generate Vβ-Dβ-Jβ combinations unique in both length and sequence.

Since leukemias and lymphomas originate from the malignant transformation of individual lymphoid cells, all leukemias and lymphomas generally share one or more cell-specific or "clonal" antigen receptor gene rearrangements. Therefore, tests that detect *TRB* clonal rearrangements can be useful in the study of B- and T-cell malignancies.

## Specimen Requirement

50 ng of high-quality genomic DNA.

## References

1. EP Rock et al., J Exp Med 179 (1): 323-8 (1994).
2. JE Miller et al., Molecular Genetic Pathology (2nd Ed., sections 30.2.7.13 and 30.2.7.18). New York, USA: Springer Science & Business Media (2013).

## Simplified Representation of the *TRB* Gene


Depicted are the variable (Vβ), diversity (Dβ), and joining (Jβ) gene regions involved in *TRB* gene rearrangements, in addition to the downstream consensus (Cβ) gene regions.

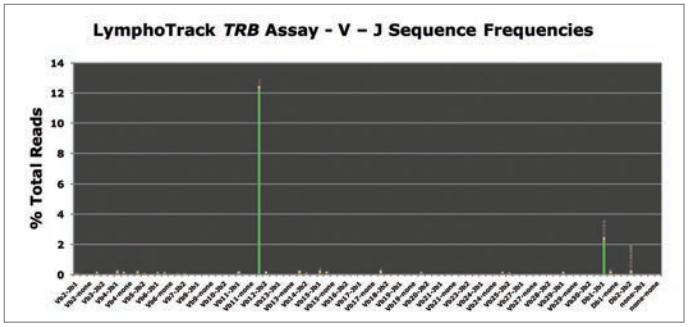
## Reagents - MiSeq® Detection

### Kit A Components

| Master Mix Name | Index # |
|---|---|
| *TRB* MiSeq 01 | A001 |
| *TRB* MiSeq 02 | A002 |
| *TRB* MiSeq 03 | A003 |
| *TRB* MiSeq 04 | A004 |
| *TRB* MiSeq 05 | A005 |
| *TRB* MiSeq 06 | A006 |
| *TRB* MiSeq 07 | A007 |
| *TRB* MiSeq 08 | A008 |
| **Controls** | |
| *TRB* POS (+) Qty. 1 | NGS NEG (-) Qty. 1 |

### Panel Components (includes all master mixes from Kit A plus the items below)

| Master Mix Name | Index # | Master Mix Name | Index # |
|---|---|---|---|
| *TRB* MiSeq 09 | A009 | *TRB* MiSeq 18 | A018 |
| *TRB* MiSeq 10 | A010 | *TRB* MiSeq 19 | A019 |
| *TRB* MiSeq 11 | A011 | *TRB* MiSeq 20 | A020 |
| *TRB* MiSeq 12 | A012 | *TRB* MiSeq 21 | A021 |
| *TRB* MiSeq 13 | A013 | *TRB* MiSeq 22 | A022 |
| *TRB* MiSeq 14 | A014 | *TRB* MiSeq 23 | A023 |
| *TRB* MiSeq 15 | A015 | *TRB* MiSeq 25 | A025 |
| *TRB* MiSeq 16 | A016 | *TRB* MiSeq 27 | A027 |
| **Controls** | | | |
| *TRB* POS (+) Qty. 3 | | NGS NEG (-) Qty. 3 | |




**V-J Sequence Frequencies.** The LymphoTrack bioinformatics software provides PDF reports which include Top 10 Merged Read Summary as well as a stacked bar graph depicting the relative frequencies of the 200 most prevalent rearrangements sequenced and identified in the sample. To learn more about the LymphoTrack software, please refer to the LymphoTrack Bioinformatics Software section.

## Ordering Information

| Catalog # | Products | Quantity Components |
|---|---|---|
| 7-225-0009 | LymphoTrack® *TRB* Assay Kit A - MiSeq® | 8 indices - 5 sequencing reactions each |
| 7-225-0019 | LymphoTrack® *TRB* Assay Panel - MiSeq® | 24 indices - 5 sequencing reactions each |
| 7-500-0009 | LymphoTrack® Software - MiSeq® | 1 CD complimentary with purchase |
| 7-500-0008 | LymphoTrack® MRD Software* | 1 CD complimentary with purchase |

*MRD Software can be used to track sequences generated by either LymphoTrack Assays - MiSeq® or Ion S5/PGM™ MRD applications are for Research Use Only. To obtain a copy, please contact your local distributor or send an e-mail to customerservice@invivoscribe.com.

# LymphoTrack Bioinformatics Software

## Software Use

The LymphoTrack Bioinformatics Software package is provided with each LymphoTrack Assay to analyze raw FASTQ files for clonality analysis of single or multiple target data sets (*IGHV* Leader (Leader), *IGH* FR1, *IGH* FR2, *IGH* FR3, *IGK, TRG, TRB*). For data generated with LymphoTrack *IGHV* Leader or *IGH* FR1 Assays the software provides additional information, including the rate of somatic hypermutation (SHM) and whether a clone will be functional based upon the presence of a premature stop codon. The software can also predict whether an open reading frame would be in- or out-of-frame, so no external data analysis is required for sample interpretation.

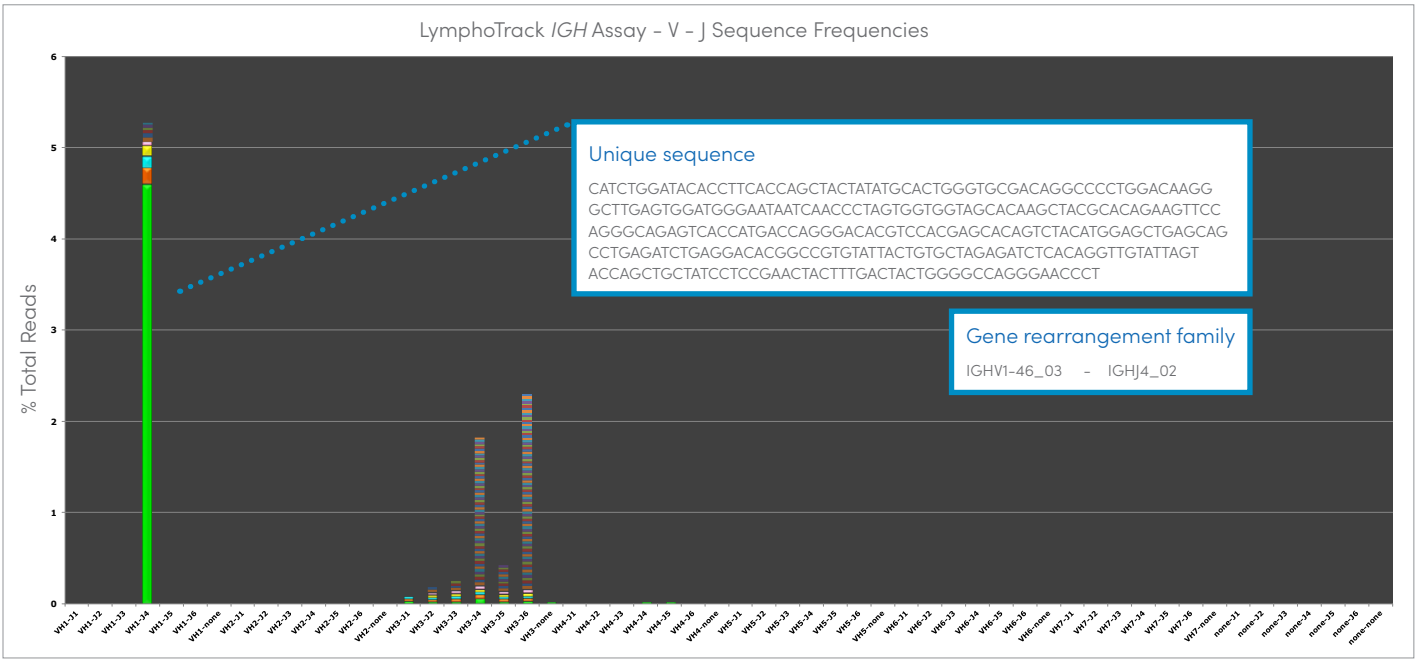
The provided software is composed of two distinct parts:

1. A bioinformatics Data Analysis Application
2. Microsoft Excel® Data Visualization spreadsheets for each gene target and automated Sample-to-PDF Reports for streamlined data analysis.

## Minimum Software Requirements

- Processor: Intel Core 2 Duo or newer CPU recommended.
- Hard Drive: At least 80 GB of free disk space is required; 250 GB recommended.
- RAM: 4 GB required; 8 GB or more recommended.
- Operating System: Windows 7 (64-bit) is required.
- Java 8 for 64-bit operating systems or higher (Java configured for 32-bit operating systems is not compatible with the LymphoTrack Software – MiSeq or LymphoTrack Software - S5/PGM). The most recent version of Java can be downloaded directly from Oracle at http://www.java.com/.
- A PDF reader, such as Adobe Acrobat Reader, to visualize data reports generated by the LymphoTrack Reporter.
- Microsoft Office Excel 2007, 2010, or 2013 with Macro support enabled is required for data visualization.
- A CD-ROM drive.
- The LymphoTrack Software - MiSeq has been validated for use with Windows 7 configured for English (United States) language and default display size settings, and Microsoft Excel 2007/2010/2013 for alternate data visualization. Use of other Windows/Excel versions and/or language/display size settings may not be compatible.
- The LymphoTrack Software - S5/PGM has been validated for use with Microsoft Excel 2007/2010/2013/2016 and Windows 7 or Windows 10 configured for English (United States) language and default display size settings, and for alternate data visualization. Use of other Windows/Excel versions and/or language/display size settings may not be compatible.

## Sequence Frequency Graph




The stacked bar graph depicts the top 200 sequencing reads for a sample. Each individual colored bar represents a unique population of cells. Different colors stacked at the same point on the x-axis represent unique sequences that utilize the same V and J gene families. The amplicons of these products vary in sequence and may also vary in product size.

## Sequencing Summary

Using the merged read summary interpretation is quick and easy.

1 Easy identification of specific types of gene rearrangements such as *IGHV*3-21.

| Rank | Sequence | Length | Merge count | V-gene | J-gene | % Total reads | Cumulative % | Mutation rate partial V-gene (%) | In-frame (Y/N) | No stop codon (Y/N) | V-coverage |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | TTCTCGTGGTG | 455 | 29603 | IGHV4-59_08 | IGHJ4_02 | 9.93 | 9.93 | 11.26 | Y | Y | 98.63 |
| 2 | CTCGCCCTCCT | 463 | 205 | IGHV5-51_01 | IGHJ4_02 | 0.07 | 9.99 | 0.00 | Y | Y | 99.66 |
| 3 | GGTTTTCCTTG | 484 | 201 | IGHV3-7_01 | IGHJ4_02 | 0.07 | 10.06 | 7.77 | Y | Y | 100.00 |
| 4 | CTCGCCCTCCT | 463 | 185 | IGHV5-51_01 | IGHJ5_02 | 0.06 | 10.12 | 6.08 | Y | Y | 99.32 |
| 5 | CTCGCCCTCCT | 469 | 170 | IGHV5-51_01 | IGHJ4_02 | 0.06 | 10.18 | 0.00 | Y | Y | 99.32 |
| 6 | CTCGCCCTCCT | 466 | 160 | IGHV5-51_01 | IGHJ4_02 | 0.05 | 10.23 | 0.00 | Y | Y | 99.66 |
| 7 | CTGCTGCTGAC | 460 | 159 | IGHV2-5_10 | IGHJ5_02 | 0.05 | 10.29 | 8.08 | Y | Y | 97.64 |
| 8 | GGTTTTCCTTG | 493 | 156 | IGHV3-48_02 | IGHJ6_02 | 0.05 | 10.34 | 3.72 | Y | Y | 98.99 |
| 9 | CTCGCCCTCCT | 334 | 153 | IGHV5-51_02 | IGHJ2_01 | 0.05 | 10.39 | 3.72 | Y | N | 27.70 |
| 10 | CTCGCCCTCCT | 334 | 152 | IGHV5-51_02 | IGHJ2_01 | 0.05 | 10.44 | 3.38 | Y | N | 26.01 |

2 Identification of clonal sequences for follow up tracking with LymphoTrack MRD Software.

3 SHM rate and indicators to determine whether a clone is productive. Only provided for *IGHV* Leader and *IGH* FR1.

The read summary provides sequences from a sample ranked from most abundant to least prevalent. The total read count for individual sequences is provided and no independent analysis is required to determine V and J gene families and predictions for SHM when analyzing data from LymphoTrack *IGHV* Leader or *IGH* FR1 Assays. Additionally, the software provides raw and merged data in which reads that differ by 1-2 bp are automatically merged to account for possible sequencing errors and to improve the accuracy and ease of sample interpretation. combined.

## Ordering Information

| Catalog # | Products | Quantity Components |
|---|---|---|
| 7-500-0009 | LymphoTrack® Software- MiSeq® | 1 CD complimentary with kit purchase |
| 7-500-0007 | LymphoTrack® Software - S5/PGM™ | 1 CD complimentary with kit purchase |

# LymphoTrack® Assays

## Minimal Residual Disease (MRD) Solution

## Key Benefits

- Complete solution for MRD clonality testing includes controls and software
- Ensures test sensitivity to enable confidence in reporting
- Facilitates standardization of clonotype tracking
- LymphoTrack MRD Software for automated analyses with PDF Reports
- Longitudinal assessment of mutation status of B- and T-cell clonality including gene rearrangements and somatic hypermutation (SHM)
- LymphoTrack Assays formatted for both Illumina® and Thermo Fisher NGS Platforms available

52/ LymphoTrack Minimal Residual Disease (MRD) Software

54/ LymphoTrack B-cell & T-cell Low Positive Controls

55/ LymphoQuant B-cell & T-cell Internal Control

LymphoTrack®
Assays

# MRD testing by Next-Generation Sequencing (NGS) is a proven tool in the development of management strategies for hematologic malignancies.

A number of investigators have described NGS-based approaches that have demonstrated success in detecting and monitoring MRD in Chronic Lymphocytic Leukemia (CLL), Acute Lymphoblastic Leukemia (ALL) and other lymphoid malignancies.[1,2] LymphoTrack Assays are NGS-based deep sequencing assays that detect virtually all clonal rearrangements within targeted T-cell receptor (TCR) or immunoglobulin (Ig) antigen receptor loci. Once a specific rearrangement (the clonotype) has been identified, LymphoTrack assays can be used to track these clonotype populations to a sensitivity as low as $10^{-6}$. Complementing the LymphoTrack Assays, the LymphoTrack MRD solution is a bundled product set for improved Minimal Residual Disease (MRD) assessment and tracking of rearrangement (clonotype) sequences.

LymphoTrack MRD B-cell and T-cell bundles offer a complete MRD solution for clonality tracking. B- or T-cell bundles may be purchased that include the LymphoTrack Assay, LymphoTrack MRD Bioinformatics Software, a LymphoTrack Low Positive Control and a LymphoQuant Internal Control. Each bundle facilitates the standardization of MRD testing by providing controls suitable for longitudinal MRD tracking with test sensitivity assurance. LymphoTrack MRD Software further simplifies clonal tracking due to rich sequence specific data analyses. This software enables longitudinal monitoring of clonal populations by providing multiple functionalities to the user including project planning features and automated bioinformatics applications.

When monitoring MRD, a highly sensitive detection method such as NGS-based LymphoTrack may aid in the early detection of lymphoproliferative disease relapse. However, MRD test results are dependent on DNA amounts interrogated, as well as the confidence level of the test. Controls tracking MRD test sensitivity are thus necessary when reporting MRD test results. Designed for MRD testing, the LymphoTrack Low Positive Control confirms the sensitivity of respective LymphoTrack MRD runs match or exceed a $10^{-4}$ (or 1 in 10,000) level. Detection of the LymphoTrack Low Positive Control thus lessens false negative reporting concerns at $10^{-4}$, and is further necessary to report MRD negative results with confidence at $10^{-4}$.

Consistent use of a spike-in internal control promotes objective monitoring of clonality over time enabling test standardization. To serve this need, Invivoscribe offers a LymphoQuant B-cell and T-cell Internal Control. Addition of a spike-in LymphoQuant B-cell or T-cell Internal Control to samples allows for the estimation of clonotype cell equivalents to facilitate longitudinal clonotype tracking over time.

References: [1] Leukemia 27:1659-1665, 2013. [2] Blood 120:5173-5180, 2012.

# LymphoTrack Minimal Residual Disease (MRD) Software

## Software Use

The LymphoTrack MRD Bioinformatics Software package is provided with each LymphoTrack Assay upon request to aid in the evaluation of treatment response in many lymphoid malignancies such as Acute Lymphoblastic Leukemia and Multiple Myeloma to minimize the risk of patients relapsing. The exceptional sensitivity and precision of NGS-based MRD tracking can accelerate clinical trials and drug development. This MRD software is intended to detect the presence of clonotype sequences within the output files generated using the Invivoscribe LymphoTrack Assays and accompanying LymphoTrack bioinformatics software; it is not intended to define the significance of these findings. Once a specific rearrangement sequence (the clonotype) has been identified in a primary sample, the MRD software enables streamlined tracking of clonal populations at a sensitivity of $10^{-4}$, or even lower limits provided sufficient DNA is tested. The MRD software can also be used for simultaneous objective tracking of bi-allelic sequences across multiple replicates for longitudinal subject tracking and in drug development studies.

The provided software is composed of three distinct parts:

1. A bioinformatics Data Analysis Application
2. A Project Planner that can be used to calculate confidence based on read depth, replicate count, and DNA input
3. PDF report identifying the clonotype sequence if present and summarizing the degree of mismatches, calculating the read frequency and the degree of confidence if a certain clonotype is not present at sensitivities of $10^{-3}$, $10^{-4}$, $10^{-5}$, and $10^{-6}$.

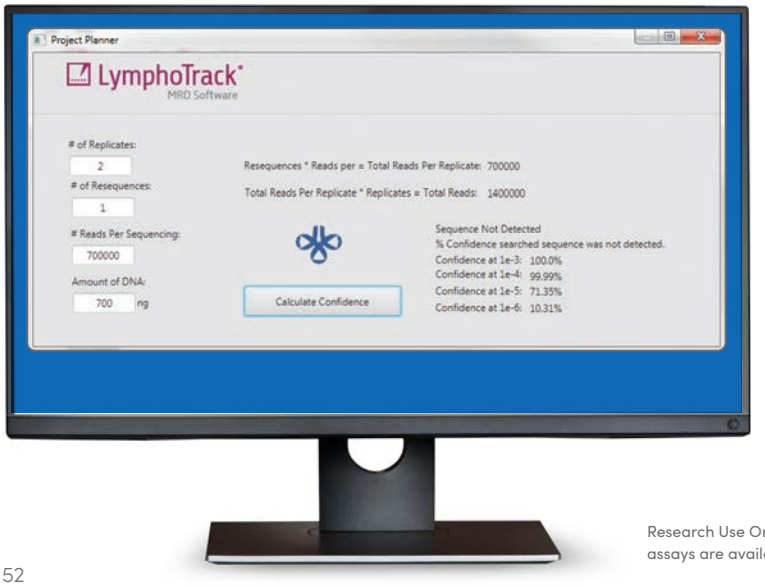
## Minimum Software Requirements

- Processor: Intel Core 2 Duo or newer CPU recommended
- Hard Drive: At least 1 GB of free disk space is required; 2 GB recommended
- RAM: 4 GB required; 8 GB or more recommended
- Operating System: Windows 7 (64-bit)
- A CD-ROM drive*

* If a CD-ROM drive is not available, please contact us at: support@invivoscribe.com

## MRD Project Planner

The Project Planner can be used to calculate the confidence of a true negative by adding replicate counts, resequencing counts, sequencing depth, and DNA input amount. The software assumes that the same sequencing depth and DNA input is used for each replicate.




Research Use Only (RUO) assays are not for sale in Europe and other global markets where equivalent CE-IVD assays are available and registered with the appropriate regulatory agencies.

## LymphoTrack MRD Specimen Report

This report summarizes the overall call, i.e. if a clonotype was detected or not detected, the number of checked replicates, the total DNA input, total reads analyzed and the location of all output files. The values in the PDF report are also found in the generated text files.

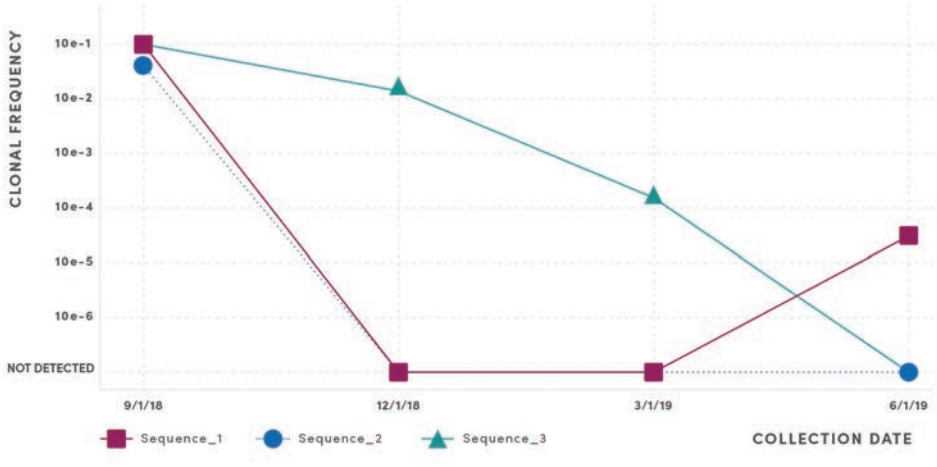
| Sequence # | 1 | Sequence Name | Sequence_1 | | |
|---|---|---|---|---|---|
| Replicate | 1 | MRD Status* | NOT DETECTED | Reads in Replicate | 950000 |

CAGCCTCTAGATTCGTCTTTGATGATGTTGTCATCCACTGACGCCACGCTCCAGGGAAGGGCCTGGAGAGACGTCTGCAGAGGACGTCGTA
GGAGCGCCTCCTAAAGAATCTTGAATACTGGGGCCGGGGAACCCTGGTCACCGTGTCCTCAGGTGAGTCCTCGTCACCGTGTCCTCACGTG
AGTCCTCAGAAACTGGGAACCCT

| Replicate Details | Cumulative Target Read Count | Cumulative % Total Reads | Cumulative LymphoQuant Read Count | Clonal Frequency |
|---|---|---|---|---|
| Exact Match | 1 | NOT DETECTED | 300 | NOT DETECTED |
| 1 Mismatch | 1 | NOT DETECTED | 300 | NOT DETECTED |
| 2 Mismatch | 1 | NOT DETECTED | 300 | NOT DETECTED |

| Detection Limit | % Confidence | Detection Limit | % Confidence |
|---|---|---|---|
| 1e-3 | 100.0% | 1e-5 | 64.3% |
| 1e-4 | 99.99% | 1e-6 | 3.53% |

## MRD Summary Graph




NOTE: The MRD Report may slightly differ from what is shown.

## Ordering Information

| Catalog # | Products | Quantity Components |
|---|---|---|
| 7-500-0008 | LymphoTrack® MRD Software** | 1 CD complimentary with LymphoTrack kit purchase |

** MRD Software can be used to track sequences generated using either LymphoTrack® Assays formatted for either the Illumina® or Thermo Fisher® NGS platforms.

MRD applications are for Research Use Only. To obtain a copy, please contact your local distributor or send an e-mail to customerservice@invivoscribe.com

# Low Positive Controls

Minimal Residual Disease (MRD) testing is a valuable tool that allows investigators to study and monitor multiple myeloma (MM) , chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (AML) and other hematologic diseases. Recent treatment advances have led to significantly increased clinical response and overall survival, but ultimately most subjects will relapse, driving the need for sensitive MRD monitoring. Sensitive and standardized testing such as NGS-based MRD may one day enable identification of those cases that will eventually relapse versus those who are potentially cured. In addition to the need for more sensitive tracking, it is clear that standardized methods are needed. Currently, MRD methods are highly subjective and recommendations are often based on consensus expert-shared knowledge and experience, not on a validated, objective method. Once specific rearrangements have been identified, LymphoTrack assays can be used with LymphoQuant and LymphoTrack Low Positive Controls to track these clonotype populations to a sensitivity as low as $10^{-4}$.

## LymphoTrack® B-cell Low Positive Control

LymphoTrack® B-cell Low Positive Control can be used as a control for:

| | |
|---|---|
| Gene Rearrangements: | *IGH, IGK* |
| Chromosome Translocations: | n/c |
| Mutations: | n/c |

| Catalog # | Description |
|---|---|
| 4-088-0098 | LymphoTrack® B-cell Low Positive Control |

## LymphoTrack® T-cell Low Positive Control

LymphoTrack® T-cell Low Positive Control can be used as a control for:

| | |
|---|---|
| Gene Rearrangements: | *TRB, TRG* |
| Chromosome Translocations: | n/c |
| Mutations: | n/c |

| Catalog # | Description |
|---|---|
| 4-088-0108 | LymphoTrack® T-cell Low Positive Control |

Note: Same product listed on page 138 in DNA controls section.

MRD applications are for Research Use Only (RUO); not intended for *in vitro* diagnostic use.

# Internal Controls

LymphoQuant T-cell or B-cell Internal Controls may be spiked into specimens to estimate the respective number of clonotype T-cell or B-cell equivalents present. Addition of the LymphoQuant Internal Control to the specimen PCR facilitates clonotype tracking over time without any additional sequencing cost. Consistent use of a LymphoQuant Internal Control enables investigators to objectively monitor the disease over time with a highly standardized, sensitive method. The LymphoTrack MRD software will help researchers that use the LymphoQuant Internal Control, calculate and report an estimated number of clonotype cell equivalents and the percent clonotype in the sample, enabling researchers and pharmaceutical companies to accurately monitor hematologic disease in longitudinal studies.

## LymphoQuant® B-cell Internal Control

LymphoQuant B-cell Internal Control can be used to objectively track Ig clonotypes.

| | |
|---|---|
| Gene Rearrangements: | *IGH, IGK* |
| Chromosome Translocations: | n/c |
| Mutations: | n/c |

| Catalog # | Description |
|---|---|
| 4-088-0118 | LymphoQuant® B-cell Internal Control |

## LymphoQuant® T-cell Internal Control

LymphoQuant T-cell Internal Control can be used to objectively track TCR clonotypes.

| | |
|---|---|
| Gene Rearrangements: | *TRB, TRG* |
| Chromosome Translocations: | n/c |
| Mutations: | n/c |

| Catalog # | Description |
|---|---|
| 4-088-0128 | LymphoQuant® T-cell Internal Control |

Note: Same product listed on page 139 in DNA controls section.

MRD applications are for Research Use Only (RUO); not intended for *in vitro* diagnostic use.

# IdentiClone®

## Gel and Capillary CE IVD Assays

IdentiClone®

# IdentiClone Assay kits are CE-marked *in vitro* diagnostic products.*

## These kits are intended for PCR-based detection of clonal gene rearrangements and translocations in patients with suspected lymphoproliferations, using gel or capillary electrophoresis methods.

These PCR-based tests include standardized Instructions For Use (IFUs) with interpretation guidelines describing the use of the kits' master mixes and controls. Master mixes are composed of a buffered magnesium chloride solution, deoxynucleotides, and multiple primers that target the gene segments of interest. Multiple primers are necessary to ensure a comprehensive testing approach that reliably identifies clonal rearrangements.
These assay master mixes are complete with the exception of Taq DNA polymerase, which is not provided.
A single thermal cycler program and similar detection methods are used within each IdentiClone kit to improve consistency, reduce human error, and facilitate cross-training.

These assays are available in regular (33 reactions) and in MegaKit formats (330 reactions).

For more information, please visit www.invivoscribe.com

*NOTICE: IdentiClone Assays are *in vitro* diagnostic products and are not available for sale or use within North America. For more information regarding the research use only reagents, please see the Gel & Capillary Research Use Only Assays section.

# *IGH + IGK* B-Cell Clonality Assays

## Assay Description

The IdentiClone *IGH + IGK* B-Cell Clonality Assays are *in vitro* diagnostic products intended for PCR-based detection of clonal immunoglobulin heavy chain and kappa light chain gene rearrangements in patients with suspect lymphoproliferations.

Specifically, the IdentiClone *IGH + IGK* B-Cell Clonality Assays can be used to:

- Identify clonality in atypical lymphoproliferative disorders
- Support a differential diagnosis between reactive lesions and hematologic malignancies
- Assign presumptive lineage in mature monoclonal lymphoproliferative disorders
- Identify tumor-specific markers (*IGH* and *IGK* gene rearrangements) for post-treatment monitoring
- Monitor and evaluate disease recurrence

## Summary and Explanation of the Test

The Invivoscribe CE-marked IdentiClone Assays represent a unique approach to PCR-based clonality testing. These standardized assays were carefully optimized, testing positive and negative control samples using multiplex master mixes. Assay development was followed by extensive validation including the testing of more than 400 clinical samples using Revised European/American Lymphoma (REAL) Classification. Testing was done at more than thirty prominent independent testing centers throughout Europe in a collaborative study known as the BIOMED-2 Concerted Action. Results from this BIOMED-2 study appear in *Leukemia*, a leading peer-reviewed journal.[1]

These kits include six master mixes to test for rearrangements of both *IGH* and *IGK*. The *IGH* Tube A, B, and C master mixes target the framework 1, 2, 3 regions (respectively) within the variable (VH) region, and the joining (JH) region of the immunoglobulin heavy chain locus. The *IGK* Tube A master mix targets the variable (Vκ) and the joining (Jκ) region. *IGK* Tube B master mix targets kappa deleting element ($K_{de}$) rearrangements with the variable (Vκ) region and the intragenic Jκ-Cκ region. The resulting Vκ-$K_{de}$ and Jκ-Cκ intron-$K_{de}$ rearrangements are a result of unsuccessful rearrangements retained by the B cell. For best sensitivity, it is recommended to test suspect B-cell malignancies for both *IGH* and *IGK*.[3] The included Specimen Control Size Ladder master mix targets multiple genes and generates a series of amplicons of approximately 100, 200, 300, 400, and 600 base pairs to ensure that the quality and quantity of input DNA is adequate to yield a valid result.

A single thermal cycler program and similar detection methodologies are used with all of our Gene Clonality Assays. This improves consistency and facilitates cross-training on a broad range of different assays.

## Performance Characteristics

Data from two independent studies that tested more than 300 patient samples of varying types suggests the diagnostic accuracy of selected IdentiClone tests to be 96%. In both peer-reviewed studies, there were no clear false-positive results generated using the IdentiClone tests, and there was a high level of precision.[2]
The clinico-histopathological diagnosis correlated well with PCR results in a higher number of patients when compared with Southern Blot (SB) results, as seen below:

| | PCR/SB concordance:[1] |
|---|---|
| *IGH*: | 93% sensitivity / 92% specificity |
| *IGK*: | 90% sensitivity / 90% specificity |
| *IGL*: | 86% sensitivity / 92% specificity |
| *TRB*: | 86% sensitivity / 98% specificity |
| *TRG*: | 89% sensitivity / 94% specificity |
| *TRD*: | 83% sensitivity / 95% specificity |

PCR vs. SB analysis relative to histopathology and final diagnosis:

| | PCR/SB concordance:[2] | PCR sensitivity: | SB sensitivity: |
|---|---|---|---|
| *IGH + IGK*: | 85% | 98% | 39% |
| *TRB*: | 85% | 96% | 35% |

## Reference

1. JJM van Dongen et al., *Leukemia* 17:2257-2317 (2003).
2. Y Sandberg et al., *J. Mol. Diag.* 7(4):495-503 (2005).
3. Van Krieken, JHJM et al., *Leukemia* 21:201 - 206 (2007).

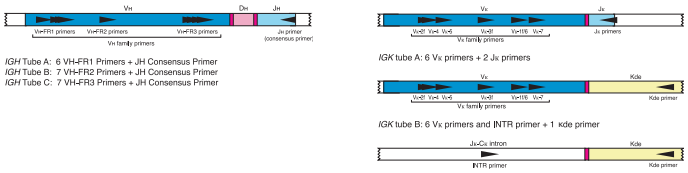



**Figure Legend:** Simple representation of the organization of a rearranged immunoglobulin heavy chain (*IGH*) gene on chromosome 14q32.33 and the immunoglobulin kappa light chain gene on chromosome 2p11.2. Black arrows represent the relative positions of primers that target the conserved framework regions (FR1-3) and the downstream consensus JH gene segments for *IGH* and the Vκ, Jκ, INTR and Kde primers which are included in the *IGK* master mix tubes.

EuroClonality

This assay is based on the **EuroClonality/BIOMED-2** Concerted Action BMH4-CT98-3936.

# IdentiClone®

*IGH + IGK* B-Cell Clonality Assays

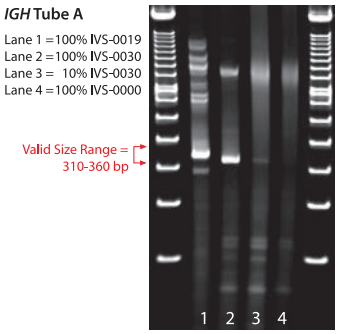
Gel and Capillary CE IVD Assays

## Reagents

| Controls | Concentration | Units in Assay | Units in Assay MegaKit |
|---|---|---|---|
| IVS-0030 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0019 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0007 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0000 Polyclonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| **Master Mixes** | **Target** | **Units in Assay** | **Units in Assay MegaKit** |
| *IGH* Tube A | Framework 1 + JH | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *IGH* Tube B | Framework 2 + JH | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *IGH* Tube C | Framework 3 + JH | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *IGK* Tube A | Vκ-Jκ | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *IGK* Tube B | Vκ-Kde, Intron-Kde | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| Specimen Control Size Ladder | Multiple Genes | 1 x 1500 µL tube | 10 x 1500 µL tubes |

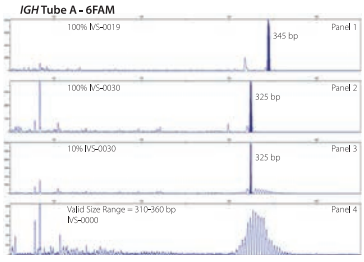
## Gel Electrophoresis Detection

Heteroduplex analysis is performed to differentiate clonal and non-clonal PCR products. It involves heat denaturation of double-stranded DNA, followed by a snap chilling process to force DNA strands to quickly reanneal. In non-clonal populations, this process causes the majority of the single-stranded DNA to incorrectly bind to non-homologous strands, resulting in secondary structures and reducing the DNA's ability to migrate through a non-denaturing polyacrylamide gel. As a result, polyclonal products are frequently observed as smears at high molecular weights. In clonal populations, after heteroduplex analysis is performed, most denatured single-strand PCR products will reanneal correctly with homologous strands of DNA (reforming homoduplexes) allowing them to easily migrate through the polyacrylamide gel as a single band. Therefore, heteroduplex analysis is key for analyzing PCR products visualized using gel detection methods, as it increases the separation between clonal and polyclonal products.




## Capillary Electrophoresis Detection (ABI)

Differential fluorescence detection, such as ABI fluorescence detection, is commonly used to resolve different-sized amplicon products using a capillary electrophoresis instrument. Primers can be conjugated with several different fluorescent dyes (fluorophores), so that they produce different emission spectra upon excitation by a laser in the capillary electrophoresis instrument. In this manner, different fluorescent dyes can correspond to different targeted regions. This detection system results in excellent sensitivity, single nucleotide resolution, differential product detection, and relative quantification. In addition, differential detection allows accurate, reproducible and objective interpretation of primer-specific products. Inter-assay and intra-assay reproducibility in size determination using capillary electrophoresis is approximately 1 to 4 nucleotides.




## Ordering Information

| Catalog # | Products | Quantity |
|---|---|---|
| 9-100-0010 | IdentiClone® *IGH* + *IGK* B-Cell Clonality Assay - Gel Detection | 33 reactions |
| 9-100-0020 | IdentiClone® *IGH* + *IGK* B-Cell Clonality Assay MegaKit - Gel Detection | 330 reactions |
| 9-100-0031 | IdentiClone® *IGH* + *IGK* B-Cell Clonality Assay - ABI Fluorescence Detection | 33 reactions |
| 9-100-0041 | IdentiClone® *IGH* + *IGK* B-Cell Clonality Assay MegaKit - ABI Fluorescence Detection | 330 reactions |

These are *in vitro* diagnostic products, and are not available for sale or use within North America.

# *IGH* Gene Clonality Assays

## Assay Description

The IdentiClone *IGH* Gene Clonality Assays are *in vitro* diagnostic products intended for PCR-based detection of clonal immunoglobulin heavy chain gene rearrangements in patients with suspect lymphoproliferations.

Specifically, the IdentiClone *IGH* Gene Clonality Assays can be used to:

- Identify clonality in atypical lymphoproliferative disorders
- Support a differential diagnosis between reactive lesions and hematologic malignancies
- Assign presumptive lineage in mature monoclonal lymphoproliferative disorders
- Identify tumor-specific markers (*IGH* gene rearrangements) for post-treatment monitoring
- Monitor and evaluate disease recurrence

## Summary and Explanation of the Test

The Invivoscribe CE-marked IdentiClone Assays represent a unique approach to PCR-based clonality testing. These standardized assays were carefully optimized, testing positive and negative control samples using multiplex master mixes. Assay development was followed by extensive validation including the testing of more than 400 clinical samples using Revised European/American Lymphoma (REAL) Classification. Testing was done at more than thirty prominent independent testing centers throughout Europe in a collaborative study known as the BIOMED-2 Concerted Action. Results from this BIOMED-2 study appear in *Leukemia*, a leading peer-reviewed journal.[1]

These test kits include six master mixes. The *IGH* Tube A, B, and C master mixes target the framework 1, 2, and 3 regions (respectively) within the variable (VH) region and the joining (JH) region of the immunoglobulin heavy chain locus. The *IGH* Tube D and E master mixes target the diversity and joining regions. The Specimen Control Size Ladder master mix targets multiple genes and generates a series of amplicons of approximately 100, 200, 300, 400, and 600 base pairs to ensure that the quality and quantity of input DNA is adequate to yield a valid result. A single thermal cycler program and similar detection methodologies are used with all of our gene clonality assays. This improves consistency and facilitates cross-training on a broad range of different assays.

## Performance Characteristics

Data from two independent studies that tested more than 300 patient samples of varying types suggests the diagnostic accuracy of selected IdentiClone tests to be 96%. In both peer-reviewed studies, there were no clear false-positive results generated using the IdentiClone tests, and there was a high level of precision.[2] The clinico-histopathological diagnosis correlated well with PCR results in a higher number of patients when compared with Southern Blot (SB) results, as seen below:

| | PCR/SB concordance:[1] |
|---|---|
| *IGH*: | 93% sensitivity / 92% specificity |
| *IGK*: | 90% sensitivity / 90% specificity |
| *IGL*: | 86% sensitivity / 92% specificity |
| *TRB*: | 86% sensitivity / 98% specificity |
| *TRG*: | 89% sensitivity / 94% specificity |
| *TRD*: | 83% sensitivity / 95% specificity |

PCR vs. SB analysis relative to histopathology and final diagnosis:

| | PCR/SB concordance:[2] | PCR sensitivity: | SB sensitivity: |
|---|---|---|---|
| *IGH + IGK*: | 85% | 98% | 39% |
| *TRB*: | 85% | 96% | 35% |

## Reference

1. JJM van Dongen et al., *Leukemia* 17:2257-2317 (2003).
2. Y Sandberg et al., *J. Mol. Diag.* 7(4):495-503 (2005).

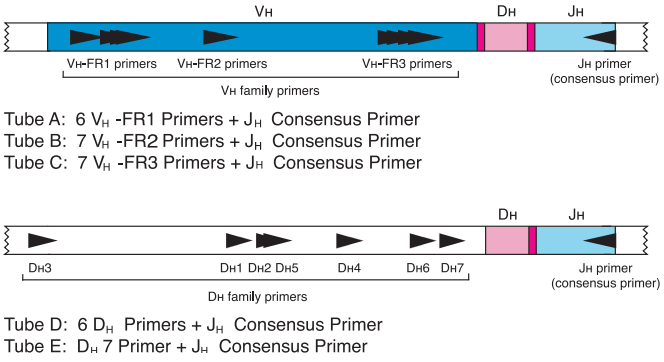



**Figure Legend:** Simple representation of the organization of a rearranged immunoglobulin heavy chain gene on chromosome 14q32.33. Black arrows represent the relative positions of primers that target the conserved framework (FR1-3) and diversity (DH1-7) regions, and the downstream consensus JH gene segments. The amplicon products generated from each of these regions can be differentially detected when fluorescent primer sets are used with capillary electrophoresis instruments that employ differential fluorescence detection.

EuroClonality This assay is based on the **EuroClonality/BIOMED-2** Concerted Action BMH4-CT98-3936.

# IdentiClone®

*IGH* Gene Clonality Assays

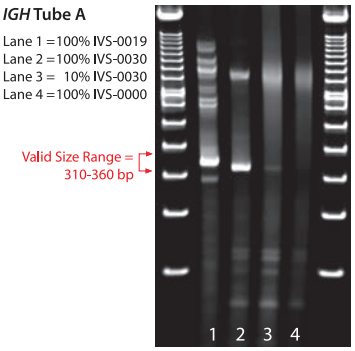
Gel and Capillary CE IVD Assays

## Reagents

| Controls | Concentration | Units in Assay | Units in Assay MegaKit |
|---|---|---|---|
| IVS-0030 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0019 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0024 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0008 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0000 Polyclonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| **Master Mixes** | **Target** | **Units in Assay** | **Units in Assay MegaKit** |
| *IGH* Tube A | Framework 1 + JH | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *IGH* Tube B | Framework 2 + JH | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *IGH* Tube C | Framework 3 + JH | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *IGH* Tube D | DH1-6 + JH | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *IGH* Tube E | DH7 + JH | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| Specimen Control Size Ladder | Multiple Genes | 1 x 1500 µL tube | 10 x 1500 µL tubes |

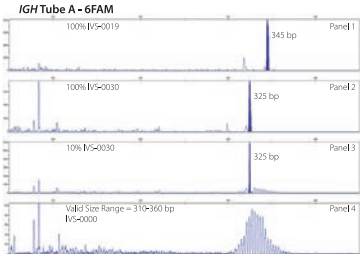
## Gel Electrophoresis Detection

Heteroduplex analysis is performed to differentiate clonal and non-clonal PCR products and involves heat denaturation of double-stranded DNA, followed by a snap chilling process to force DNA strands to quickly reanneal. In non-clonal populations, this process causes a majority of the single-stranded DNA to incorrectly bind to non-homologous strands resulting in secondary structures and reducing the DNA's ability to migrate through a non-denaturing polyacrylamide gel. As a result, polyclonal products are frequently observed as smears at high molecular weights. In clonal populations, after heteroduplex analysis is performed, most denatured single-strand PCR products will reanneal correctly with homologous strands of DNA (reforming homoduplexes) allowing them to easily migrate through the polyacrylamide gel as a single band. Therefore, heteroduplex analysis is key for analyzing PCR products visualized using gel detection methods as it increases the separation between clonal and polyclonal products.




## Capillary Electrophoresis Detection (ABI)

Differential fluorescence detection, such as ABI fluorescence detection, is commonly used to resolve different-sized amplicon products using a capillary electrophoresis instrument. Primers can be conjugated with several different fluorescent dyes (fluorophores), so that they produce different emission spectra upon excitation by a laser in the capillary electrophoresis instrument. In this manner, different fluorescent dyes can correspond to different targeted regions. This detection system results in excellent sensitivity, single nucleotide resolution, differential product detection, and relative quantification. In addition, differential detection allows accurate, reproducible and objective interpretation of primer-specific products. Inter-assay and intra-assay reproducibility in size determination using capillary electrophoresis is approximately 1 to 4 nucleotides.




## Ordering Information

| Catalog # | Products | Quantity |
|---|---|---|
| 9-101-0020 | IdentiClone® *IGH* Gene Clonality Assay - Gel Detection | 33 reactions |
| 9-101-0040 | IdentiClone® *IGH* Gene Clonality Assay MegaKit - Gel Detection | 330 reactions |
| 9-101-0061 | IdentiClone® *IGH* Gene Clonality Assay - ABI Fluorescence Detection | 33 reactions |
| 9-101-0081 | IdentiClone® *IGH* Gene Clonality Assay MegaKit - ABI Fluorescence Detection | 330 reactions |

These are *in vitro* diagnostic products, and are not available for sale or use within North America.

# *IGK* Gene Clonality Assays

## Assay Description

The IdentiClone *IGK* Gene Clonality Assays are *in vitro* diagnostic products intended for PCR-based detection of clonal immunoglobulin kappa light chain gene rearrangements in patients with suspect lymphoproliferations.

Specifically, the IdentiClone *IGK* Gene Clonality Assays can be used to:

- Identify clonality in atypical lymphoproliferative disorders
- Support a differential diagnosis between reactive lesions and hematologic malignancies
- Assign presumptive lineage in mature monoclonal lymphoproliferative disorders
- Identify tumor-specific markers (*IGK* and *IGK*-$K_{de}$ rearrangements) for post-treatment monitoring
- Monitor and evaluate disease recurrence

## Summary and Explanation of the Test

The Invivoscribe CE-marked IdentiClone Assays represent a unique approach to PCR-based clonality testing. These standardized assays were carefully optimized testing positive and negative control samples using multiplex master mixes. Assay development was followed by extensive validation including the testing of more than 400 clinical samples using Revised European/American Lymphoma (REAL) Classification. Testing was done at more than thirty prominent independent testing centers throughout Europe in a collaborative study known as the BIOMED-2 Concerted Action. Results from this BIOMED-2 study appear in *Leukemia*, a leading peer-reviewed journal.[1]

These test kits include three master mixes. The *IGK* Tube A master mix targets the variable (Vκ) and the joining (Jκ) regions of the immunoglobulin kappa light chain locus, whereas the *IGK* Tube B master mix targets kappa deleting element ($K_{de}$) rearrangements with the Vκ regions and the intragenic Jκ-Cκ regions. The Vκ-$K_{de}$ and Jκ-Cκ intron-$K_{de}$ rearrangements are a result of unsuccessful rearrangements retained by the B cell. The third master mix, the Specimen Control Size Ladder, targets multiple genes and generates a series of amplicons of approximately 100, 200, 300, 400, and 600 base pairs to ensure that the quality and quantity of input DNA is adequate to yield a valid result. A single thermal cycler program and similar detection methodologies are used with all of our gene clonality assays. This improves consistency and facilitates cross-training.

## Performance Characteristics

Data from two independent studies that tested more than 300 patient sample of varying types suggests the diagnostic accuracy of selected IdentiClone tests to be 96%. In both peer-reviewed studies, there were no clear false-positive results generated using the IdentiClone tests, and there was a high level of precision.[2] The clinico-histopathological diagnosis correlated well with PCR results in a higher number of patients when compared with Southern Blot (SB) results, as seen below:

| | PCR/SB concordance:[1] |
|---|---|
| *IGH*: | 93% sensitivity / 92% specificity |
| *IGK*: | 90% sensitivity / 90% specificity |
| *IGL*: | 86% sensitivity / 92% specificity |
| *TRB*: | 86% sensitivity / 98% specificity |
| *TRG*: | 89% sensitivity / 94% specificity |
| *TRD*: | 83% sensitivity / 95% specificity |

PCR vs. SB analysis relative to histopathology and final diagnosis:

| | PCR/SB concordance:[2] | PCR sensitivity: | SB sensitivity: |
|---|---|---|---|
| *IGH* + *IGK*: | 85% | 98% | 39% |
| *TRB*: | 85% | 96% | 35% |

## Reference

1. JJM van Dongen et al., *Leukemia* 17:2257-2317 (2003).
2. Y Sandberg et al., *J. Mol. Diag.* 7(4):495-503 (2005).

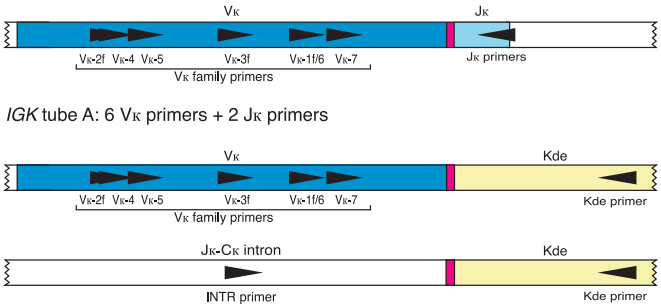



**Figure Legend:** Schematic diagram of the immunoglobulin kappa light chain gene complex on chromosome 2p11.2. Shown are the relative positions and orientations for the Vκ-Jκ, and $K_{de}$ primers, which are included in the *IGK* master mix tubes.

EuroClonality This assay is based on the **EuroClonality/BIOMED-2** Concerted Action BMH4-CT98-3936.

# IdentiClone®

*IGK* Gene Clonality Assays

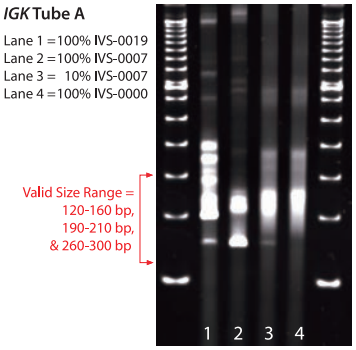
Gel and Capillary CE IVD Assays

## Reagents

| Controls | Concentration | Units in Assay | Units in Assay MegaKit |
|---|---|---|---|
| IVS-0007 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0000 Polyclonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| **Master Mixes** | **Target** | **Units in Assay** | **Units in Assay MegaKit** |
| *IGK* Tube A | $V_K$ - $J_K$ | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *IGK* Tube B | $V_K$-$K_{de}$, Intron-$K_{de}$ | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| Specimen Control Size Ladder | Multiple Genes | 1 x 1500 µL tube | 10 x 1500 µL tubes |

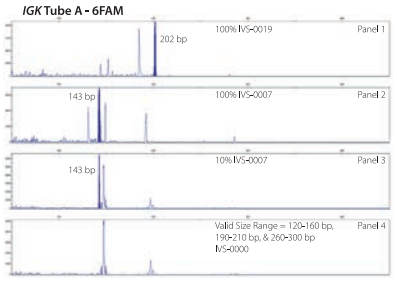
## Gel Electrophoresis Detection

Heteroduplex analysis is performed to differentiate clonal and non-clonal PCR products. It involves heat denaturation of double-stranded DNA, followed by a snap chilling process to force DNA strands to quickly reanneal. In non-clonal populations, this process causes the majority of the single-stranded DNA to incorrectly bind to non-homologous strands, resulting in secondary structures and reducing the DNA's ability to migrate through a non-denaturing polyacrylamide gel. As a result, polyclonal products are frequently observed as smears at high molecular weights. In clonal populations, after heteroduplex analysis is performed, most denatured single-strand PCR products will reanneal correctly with homologous strands of DNA (reforming homoduplexes), allowing them to easily migrate through the polyacrylamide gel as a single band. Therefore, heteroduplex analysis is key for analyzing PCR products visualized using gel detection methods, as it increases the separation between clonal and polyclonal products.




## Capillary Electrophoresis Detection (ABI)

Differential fluorescence detection, such as ABI fluorescence detection, is commonly used to resolve different-sized amplicon products using a capillary electrophoresis instrument. Primers can be conjugated with several different fluorescent dyes (fluorophores), so that they produce different emission spectra upon excitation by a laser in the capillary electrophoresis instrument. In this manner, different fluorescent dyes can correspond to different targeted regions. This detection system results in excellent sensitivity, single nucleotide resolution, differential product detection, and relative quantification. In addition, differential detection allows accurate, reproducible and objective interpretation of primer-specific products. Inter-assay and intra-assay reproducibility in size determination using capillary electrophoresis is approximately 1 to 4 nucleotides.




## Ordering Information

| Catalog # | Products | Quantity |
|---|---|---|
| 9-102-0020 | IdentiClone® *IGK* Gene Clonality Assay - Gel Detection | 33 reactions |
| 9-102-0030 | IdentiClone® *IGK* Gene Clonality Assay MegaKit - Gel Detection | 330 reactions |
| 9-102-0021 | IdentiClone® *IGK* Gene Clonality Assay - ABI Fluorescence Detection | 33 reactions |
| 9-102-0031 | IdentiClone® *IGK* Gene Clonality Assay MegaKit - ABI Fluorescence Detection | 330 reactions |

These are *in vitro* diagnostic products, and are not available for sale or use within North America.

IdentiClone®

*IGL* Gene Clonality Assays

Gel and Capillary CE IVD Assays

# *IGL* Gene Clonality Assays

## Assay Description

The IdentiClone *IGL* Gene Clonality Assays are *in vitro* diagnostic products intended for PCR-based detection of clonal immunoglobulin lambda light chain gene rearrangements in patients with suspect lymphoproliferations.

Specifically, the IdentiClone *IGL* Gene Clonality Assays can be used to:

- Identify clonality in atypical lymphoproliferative disorders
- Support a differential diagnosis between reactive lesions and hematologic malignancies
- Assign presumptive lineage in mature monoclonal lymphoproliferative disorders
- Identify tumor-specific markers (*IGL* gene rearrangements) for post-treatment monitoring
- Monitor and evaluate disease recurrence

## Summary and Explanation of the Test

The Invivoscribe CE-marked IdentiClone Assays represent a unique approach to PCR-based clonality testing. These standardized assays were carefully optimized testing positive and negative control samples using multiplex master mixes. Assay development was followed by extensive validation including the testing of more than 400 clinical samples using Revised European/American Lymphoma (REAL) Classification. Testing was done at more than thirty prominent independent testing centers throughout Europe in a collaborative study known as the BIOMED-2 Concerted Action. Results from this BIOMED-2 study appear in *Leukemia*, a leading peer-reviewed journal.[1]

These test kits include two master mixes. The *IGL* Tube master mix targets the variable (Vλ) region and the joining (Jλ) region of the immunoglobulin lambda light chain gene locus (*IGL*). The Specimen Control Size Ladder targets multiple genes and generates a series of amplicons of approximately 100, 200, 300, 400, and 600 base pairs to ensure that the quality and quantity of input DNA is adequate to yield a valid result. A single thermal cycler program and similar detection methodologies are used with all of our gene clonality assays. This improves consistency and facilitates cross-training.

## Performance Characteristics

Data from two independent studies that tested more than 300 patient samples of varying types suggests the diagnostic accuracy of selected IdentiClone tests to be 96%. In both peer-reviewed studies, there were no clear false-positive results generated using the IdentiClone tests, and there was a high level of precision.[2] The clinico-histopathological diagnosis correlated well with PCR results in a higher number of patients when compared with Southern Blot (SB) results, as seen below:

| | PCR/SB concordance:[1] |
|---|---|
| *IGH*: | 93% sensitivity / 92% specificity |
| *IGK*: | 90% sensitivity / 90% specificity |
| *IGL*: | 86% sensitivity / 92% specificity |
| *TRB*: | 86% sensitivity / 98% specificity |
| *TRG*: | 89% sensitivity / 94% specificity |
| *TRD*: | 83% sensitivity / 95% specificity |

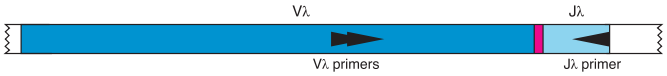
PCR vs. SB analysis relative to histopathology and final diagnosis:

| | PCR/SB concordance:[2] | PCR sensitivity: | SB sensitivity: |
|---|---|---|---|
| *IGH* + *IGK*: | 85% | 98% | 39% |
| *TRB*: | 85% | 96% | 35% |

## Reference


1. JJM van Dongen et al., *Leukemia* 17:2257-2317 (2003).

2. Y Sandberg et al., *J. Mol. Diag.* 7(4):495-503 (2005).


*IGL* tube: 2 Vλ primers + 1 Jλ primer

**Figure Legend:** Schematic diagram of the immunoglobulin lambda light chain gene complex on chromosome 22q11.2. Shown are the relative positions and orientations for the Vλ and Jλ primers, which are included in the *IGL* master mix tube. The two Vλ primers only target Vλ1, 2, and 3 because these three V families cover approximately 70% of rearrangeable Vλ gene segments, and approximately 90% of all *IGL* gene rearrangements involve these three families. Similarly, the single Jλ primer only targets Jλ1, 2, and 3 because these three J segments are involved in 98% of all *IGL* gene rearrangements.

EuroClonality

This assay is based on the **EuroClonality/BIOMED-2** Concerted Action BMH4-CT98-3936.

# IdentiClone®

*IGL* Gene Clonality Assays

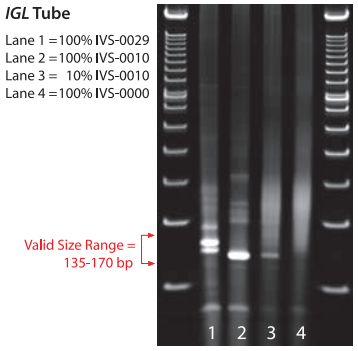
Gel and Capillary CE IVD Assays

## Reagents

| Controls | Concentration | Units in Assay | Units in Assay MegaKit |
|---|---|---|---|
| IVS-0010 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0029 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0000 Polyclonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| **Master Mixes** | **Target** | **Units in Assay** | **Units in Assay MegaKit** |
| *IGL* Tube | Vλ -Jλ | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| Specimen Control Size Ladder | Multiple Genes | 1 x 1500 µL tube | 10 x 1500 µL tubes |

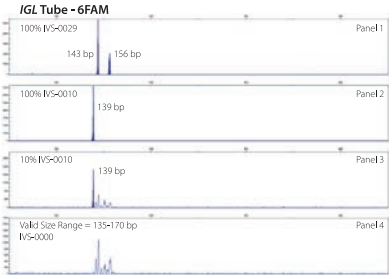
## Gel Electrophoresis Detection

Heteroduplex analysis is performed to differentiate clonal and non-clonal PCR products It involves heat denaturation of double-stranded DNA, followed by a snap chilling process to force DNA strands to quickly reanneal. In non-clonal populations, this process causes a majority of the single-stranded DNA to incorrectly bind to non-homologous strands, resulting in secondary structures and reducing the DNA's ability to migrate through a non-denaturing polyacrylamide gel. As a result, polyclonal products are frequently observed as smears at high molecular weights. In clonal populations, after heteroduplex analysis is performed, most denatured single-strand PCR products will reanneal correctly with homologous strands of DNA (reforming homoduplexes), allowing them to easily migrate through the polyacrylamide gel as a single band. Therefore, heteroduplex analysis is key for analyzing PCR products visualized using gel detection methods, as it increases the separation between clonal and polyclonal products.




## Capillary Electrophoresis Detection (ABI)

Differential fluorescence detection, such as ABI fluorescence detection, is commonly used to resolve different-sized amplicon products using a capillary electrophoresis instrument. Primers can be conjugated with several different fluorescent dyes (fluorophores), so that they produce different emission spectra upon excitation by a laser in the capillary electrophoresis instrument. In this manner, different fluorescent dyes can correspond to different targeted regions. This detection system results in excellent sensitivity, single nucleotide resolution, differential product detection, and relative quantification. In addition, differential detection allows accurate, reproducible and objective interpretation of primer-specific products. Inter-assay and intra-assay reproducibility in size determination using capillary electrophoresis is approximately 1 to 4 nucleotides.




## Ordering Information

| Catalog # | Products | Quantity |
|---|---|---|
| 9-103-0010 | IdentiClone® *IGL* Gene Clonality Assay - Gel Detection | 33 reactions |
| 9-103-0020 | IdentiClone® *IGL* Gene Clonality Assay MegaKit - Gel Detection | 330 reactions |
| 9-103-0011 | IdentiClone® *IGL* Gene Clonality Assay - ABI Fluorescence Detection | 33 reactions |
| 9-103-0021 | IdentiClone® *IGL* Gene Clonality Assay MegaKit - ABI Fluorescence Detection | 330 reactions |

These are *in vitro* diagnostic products, and are not available for sale or use within North America.

# *TCRB* + *TCRG* T-Cell Clonality Assays

## Assay Description

The IdentiClone *TCRB* + *TCRG* T-Cell Clonality Assays are *in vitro* diagnostic products intended for PCR-based detection of clonal T-cell receptor beta and gamma chain gene rearrangements in patients with suspect lymphoproliferations.

Specifically, the IdentiClone *TCRB* + *TCRG* T-Cell Clonality Assays can be used to:

- Identify clonality in suspected lymphoproliferations
- Support a differential diagnosis between reactive lesions and T-cell and some immature B-cell malignancies
- Determine lineage involvement in mature lymphoproliferative disorders
- Monitor and evaluate disease recurrence

## Summary and Explanation of the Test

The Invivoscribe CE-marked IdentiClone Assays represent a unique approach to PCR-based clonality testing. These standardized assays were carefully optimized testing positive and negative control samples using multiplex master mixes. Assay development was followed by extensive validation including the testing of more than 400 clinical samples using Revised European/American Lymphoma (REAL) Classification. Testing was done at more than thirty prominent independent testing centers throughout Europe in a collaborative study known as the BIOMED-2 Concerted Action. Results from this BIOMED-2 study appear in *Leukemia*, a leading peer-reviewed journal.[1]

These kits include six master mixes to test for rearrangements of both *TRB* (formerly known as *TCRB*) and *TRG* (formerly known as *TCRG*). *TCRB* Tubes A and B target framework regions within the variable region, and the joining region of the *TCR* beta chain locus. *TCRB* Tube C targets the diversity and joining regions of the *TCR* beta chain locus. *TCRG* Tube A contains primers that target the Vγ1-8 + Vγ10 genes and Jγ1.1, Jγ1.3, Jγ2.1, and Jγ2.3 genes (also known as JγP1, Jγ1, JγP2, and Jγ2 respectively). *TCRG* Tube B contains primers that target the Vγ9 + Vγ11 genes and Jγ1.1, Jγ1.3, Jγ2.1, and Jγ2.3 genes. For best sensitivity it is recommended to test suspect T-cell malignancies for both *TRG* and *TRB*.[3] The Specimen Control Size Ladder master mix included targets multiple genes and generates a series of amplicons of approximately 100, 200, 300, 400, and 600 base pairs to ensure that the quality and quantity of input DNA is adequate to yield a valid result. A single thermal cycler program and similar detection methodologies are used with all of our gene clonality assays. This improves consistency and facilitates cross-training on a broad range of different assays.

## Performance Characteristics

Data from two independent studies that tested more than 300 patient samples of varying types suggests the diagnostic accuracy of selected IdentiClone tests to be 96%. In both peer-reviewed studies, there were no clear false-positive results generated using the IdentiClone tests, and there was a high level of precision.[2] The clinico-histopathological diagnosis correlated well with PCR results in a higher number of patients when compared with Southern Blot (SB) results, as seen below:

| | PCR/SB concordance:[1] |
|---|---|
| *IGH*: | 93% sensitivity / 92% specificity |
| *IGK*: | 90% sensitivity / 90% specificity |
| *IGL*: | 86% sensitivity / 92% specificity |
| *TRB*: | 86% sensitivity / 98% specificity |
| *TRG*: | 89% sensitivity / 94% specificity |
| *TRD*: | 83% sensitivity / 95% specificity |

PCR vs. SB analysis relative to histopathology and final diagnosis:

| | PCR/SB concordance:[2] | PCR sensitivity: | SB sensitivity: |
|---|---|---|---|
| *IGH* + *IGK*: | 85% | 98% | 39% |
| *TRB*: | 85% | 96% | 35% |

## Reference

1. JJM van Dongen et al., *Leukemia* 17:2257-2317 (2003).
2. Y Sandberg et al., *J. Mol. Diag.* 7(4):495-503 (2005).
3. Van Krieken, JHJM et al., *Leukemia* 21:201 - 206 (2007).

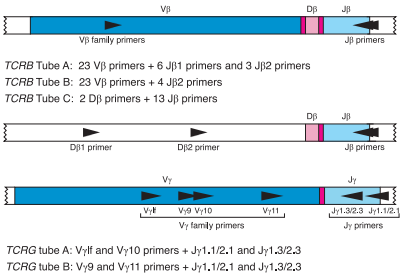



**Figure Legend:** Simplified diagram of a representative rearranged T-cell receptor beta gene and the T-cell receptor gamma gene showing the approximate placement of the upstream and downstream DNA primers. The numbers of primers and their specificity are listed for master mix *TCRB* Tubes A, B, and C and TCRG Tubes A and B. (The Vγ1f primer is a consensus primer that targets Vγ1 through Vγ8).

EuroClonality This assay is based on the **EuroClonality/BIOMED-2** Concerted Action BMH4-CT98-3936.

# IdentiClone®

*TCRB + TCRG* T-Cell Clonality Assays

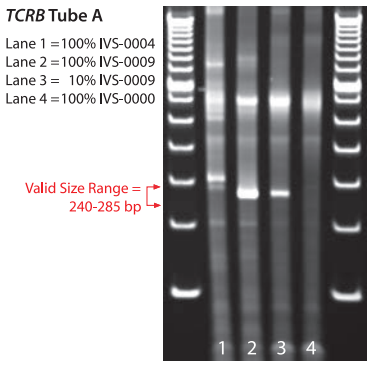
Gel and Capillary CE IVD Assays

## Reagents

| Controls | Concentration | Units in Assay | Units in Assay MegaKit |
|---|---|---|---|
| IVS-0009 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0004 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0021 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0000 Polyclonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| **Master Mixes** | **Target** | **Units in Assay** | **Units in Assay MegaKit** |
| *TCRB* Tube A | Multiple Vβ + Jβ1/2 | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *TCRB* Tube B | Multiple Vβ + Jβ2 | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *TCRB* Tube C | Multiple Dβ + Jβ1/2 | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *TCRG* Tube A | Vγβ1-8 + Vγ10 + Jγ | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *TCRG* Tube B | Vγ9 + Vγ11 + Jγ | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| Specimen Control Size Ladder | Multiple Genes | 1 x 1500 µL tube | 10 x 1500 µL tubes |

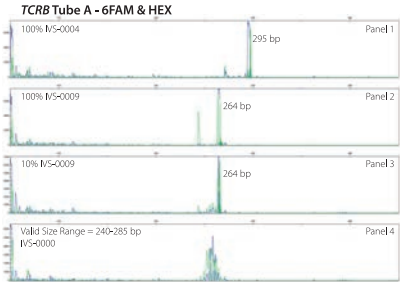
## Gel Electrophoresis Detection

Heteroduplex analysis is performed to differentiate clonal and non-clonal PCR products. It involves heat denaturation of double-stranded DNA, followed by a snap chilling process to force DNA strands to quickly reanneal. In non-clonal populations, this process causes a majority of the single-stranded DNA to incorrectly bind to non-homologous strands, resulting in secondary structures and reducing the DNA's ability to migrate through a non-denaturing polyacrylamide gel. As a result, polyclonal products are frequently observed as smears at high molecular weights. In clonal populations, after heteroduplex analysis is performed, most denatured single-strand PCR products will reanneal correctly with homologous strands of DNA (reforming homoduplexes), allowing them to easily migrate through the polyacrylamide gel as a single band. Therefore, heteroduplex analysis is key for analyzing PCR products visualized using gel detection methods, as it increases the separation between clonal and polyclonal products.




## Capillary Electrophoresis Detection (ABI)

Differential fluorescence detection, such as ABI fluorescence detection, is commonly used to resolve different-sized amplicon products using a capillary electrophoresis instrument. Primers can be conjugated with several different fluorescent dyes (fluorophores), so that they produce different emission spectra upon excitation by a laser in the capillary electrophoresis instrument. In this manner, different fluorescent dyes can correspond to different targeted regions. This detection system results in excellent sensitivity, single nucleotide resolution, differential product detection, and relative quantification. In addition, differential detection allows accurate, reproducible and objective interpretation of primer-specific products. Inter-assay and intra-assay reproducibility in size determination using capillary electrophoresis is approximately 1 to 4 nucleotides.




## Ordering Information

| Catalog # | Products | Quantity |
|---|---|---|
| 9-200-0010 | IdentiClone® *TCRB* + *TCRG* T-Cell Clonality Assay - Gel Detection | 33 reactions |
| 9-200-0020 | IdentiClone® *TCRB* + *TCRG* T-Cell Clonality Assay MegaKit - Gel Detection | 330 reactions |
| 9-200-0011 | IdentiClone® *TCRB* + *TCRG* T-Cell Clonality Assay - ABI Fluorescence Detection | 33 reactions |
| 9-200-0021 | IdentiClone® *TCRB* + *TCRG* T-Cell Clonality Assay MegaKit - ABI Fluorescence Detection | 330 reactions |

These are *in vitro* diagnostic products, and are not available for sale or use within North America.

# *TCRB* Gene Clonality Assays

## Assay Description

The IdentiClone *TCRB* Gene Clonality Assays are *in vitro* diagnostic products intended for PCR-based detection of clonal T-cell receptor beta chain gene rearrangements in patients with suspect lymphoproliferations.

Specifically, the IdentiClone *TCRB* Gene Clonality Assay can be used to:

- Identify clonality in suspected lymphoproliferations
- Support a differential diagnosis between reactive lesions and T-cell and some immature B-cell malignancies
- Determine lineage involvement in mature lymphoproliferative disorders
- Monitor and evaluate disease recurrence

## Summary and Explanation of the Test

The Invivoscribe CE-marked IdentiClone Assays represent a unique approach to PCR-based clonality testing. These standardized assays were carefully optimized testing positive and negative control samples using multiplex master mixes. Assay development was followed by extensive validation including the testing of more than 400 clinical samples using Revised European/American Lymphoma (REAL) Classification. Testing was done at more than thirty prominent independent testing centers throughout Europe in a collaborative study known as the BIOMED-2 Concerted Action. Results from this BIOMED-2 study appear in *Leukemia*, a leading peer-reviewed journal.[1]

These kits include four master mixes. *TCRB* Tubes A and B target framework regions within the variable region, and the joining region (Vβ) of the *TCR* beta chain locus. *TCRB* Tube C targets the diversity and joining (Jβ) regions of the *TCR* beta chain locus. The Specimen Control Size Ladder master mix included targets multiple genes and generates a series of amplicons of approximately 100, 200, 300, 400, and 600 base pairs to ensure that the quality and quantity of input DNA is adequate to yield a valid result. A single thermal cycler program and similar detection methodologies are used with all of our Gene Clonality Assays. This improves consistency and facilitates cross-training on a broad range of different assays.

## Performance Characteristics

Data from two independent studies that tested more than 300 patient samples of varying types suggests the diagnostic accuracy of selected IdentiClone tests to be 96%. In both peer-reviewed studies, there were no clear false-positive results generated using the IdentiClone tests, and there was a high level of precision.[2] The clinico-histopathological diagnosis correlated well with PCR results in a higher number of patients when compared with Southern Blot (SB) results, as seen below:

| | PCR/SB concordance:[1] |
|---|---|
| *IGH*: | 93% sensitivity / 92% specificity |
| *IGK*: | 90% sensitivity / 90% specificity |
| *IGL*: | 86% sensitivity / 92% specificity |
| *TRB*: | 86% sensitivity / 98% specificity |
| *TRG*: | 89% sensitivity / 94% specificity |
| *TRD*: | 83% sensitivity / 95% specificity |

PCR vs. SB analysis relative to histopathology and final diagnosis:

| | PCR/SB concordance:[2] | PCR sensitivity: | SB sensitivity: |
|---|---|---|---|
| *IGH* + *IGK*: | 85% | 98% | 39% |
| *TRB*: | 85% | 96% | 35% |

## Reference

1. JJM van Dongen et al., *Leukemia* 17:2257-2317 (2003).

2. Y Sandberg et al., *J. Mol. Diag.* 7(4):495-503 (2005).


Tube A: 23 Vβ primers + 6 Jβ1 primers and 3 Jβ2 primers
Tube B: 23 Vβ primers + 4 Jβ2 primers
Tube C: 2 Dβ primers + 13 Jβ primers

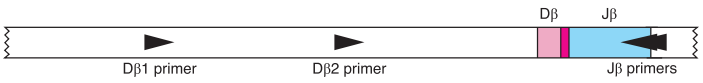



**Figure Legend:** Simplified diagram of a representative rearranged T-cell receptor beta gene showing the approximate placement of the upstream and downstream DNA primers. The numbers of primers and their specificity are listed for Master Mix Tubes A, B, and C.

EuroClonality This assay is based on the **EuroClonality/BIOMED-2** Concerted Action BMH4-CT98-3936.

# IdentiClone®

*TCRB* Gene Clonality Assays

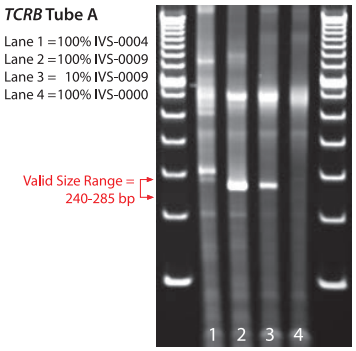
Gel and Capillary CE IVD Assays

## Reagents

| Controls | Concentration | Units in Assay | Units in Assay MegaKit |
|---|---|---|---|
| IVS-0009 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0004 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0000 Polyclonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0021 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| **Master Mixes** | **Target** | **Units in Assay** | **Units in Assay MegaKit** |
| *TCRB* Tube A | Multiple Vβ + Jβ1/2 | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *TCRB* Tube B | Multiple Vβ + Jβ2 | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *TCRB* Tube C | Multiple Dβ + Jβ1/2 | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| Specimen Control Size Ladder | Multiple Genes | 1 x 1500 µL tube | 10 x 1500 µL tubes |

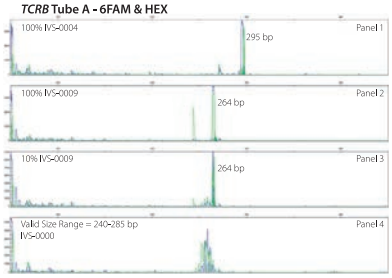
## Gel Electrophoresis Detection

Heteroduplex analysis is performed to differentiate clonal and non-clonal PCR products. It involves heat denaturation of double-stranded DNA, followed by a snap chilling process to force DNA strands to quickly reanneal. In non-clonal populations, this process causes a majority of the single-stranded DNA to incorrectly bind to non-homologous strands, resulting in secondary structures and reducing the DNA's ability to migrate through a non-denaturing polyacrylamide gel. As a result, polyclonal products are frequently observed as smears at high molecular weights. In clonal populations, after heteroduplex analysis is performed, most denatured single-strand PCR products will reanneal correctly with homologous strands of DNA (reforming homoduplexes), allowing them to easily migrate through the polyacrylamide gel as a single band. Therefore, heteroduplex analysis is key for analyzing PCR products visualized using gel detection methods, as it increases the separation between clonal and polyclonal products.




## Capillary Electrophoresis Detection (ABI)

Differential fluorescence detection, such as ABI fluorescence detection, is commonly used to resolve different-sized amplicon products using a capillary electrophoresis instrument. Primers can be conjugated with several different fluorescent dyes (fluorophores), so that they produce different emission spectra upon excitation by a laser in the capillary electrophoresis instrument. In this manner, different fluorescent dyes can correspond to different targeted regions. This detection system results in excellent sensitivity, single nucleotide resolution, differential product detection, and relative quantification. In addition, differential detection allows accurate, reproducible and objective interpretation of primer-specific products. Inter-assay and intra-assay reproducibility in size determination using capillary electrophoresis is approximately 1 to 4 nucleotides.




## Ordering Information

| Catalog # | Products | Quantity |
|---|---|---|
| 9-205-0010 | IdentiClone® *TCRB* Clonality Assay - Gel Detection | 33 reactions |
| 9-205-0020 | IdentiClone® *TCRB* Clonality Assay MegaKit - Gel Detection | 330 reactions |
| 9-205-0011 | IdentiClone® *TCRB* Clonality Assay - ABI Fluorescence Detection | 33 reactions |
| 9-205-0021 | IdentiClone® *TCRB* Clonality Assay MegaKit - ABI Fluorescence Detection | 330 reactions |

These are *in vitro* diagnostic products, and are not available for sale or use within North America.

# T-Cell Receptor Gamma Gene Rearrangement Assay 2.0

## Assay Description

The IdentiClone T-Cell Receptor Gamma Gene Rearrangement Assay 2.0 is an *in vitro* diagnostic product intended for PCR-based detection of clonal T-cell receptor gamma chain gene rearrangements in patients with suspect lymphoproliferations.

Specifically, the T-Cell Receptor Gamma Gene Rearrangement Assay 2.0 can be used to identify clonality in suspected lymphoproliferations.

## Summary and Explanation of the Test

Rearrangements of the antigen receptor genes occur during ontogeny in B- and T-lymphocytes. These gene rearrangements generate products that are unique in length and sequence. Polymerase chain reaction (PCR) assays can be used to identify lymphocyte populations derived from a single cell by detecting the unique V-J gene rearrangements present within these antigen receptor loci[1].

The Invivoscribe designed CE-marked IdentiClone Assays represents an improved approach to PCR-based clonality testing of lymphoproliferative disorders, as it can detect the vast majority of T-cell receptor gamma (*TRG*, formerly known as *TCRG*) gene rearrangements with a single multiplex master mix.

This assay allows for amplification of the *TRG* region with fluorescent labeled primers, yielding products that can be grouped under a single Gaussian distribution when separated by size using capillary electrophoresis. In addition, the product size facilitates increased success when testing FFPE samples. The included analysis algorithm aids in the interpretation of data and identification of significant clonal peaks. Presence or absence of molecular clonality can support the differential diagnosis of reactive lesions and certain B- and T-cell malignancies, provided that the results are interpreted in the context of all available clinical, histological, and immunophenotypic data.

## Performance Characteristics

To assess the performance of the *TCRG* 2.0 Assay, testing was performed on cell lines with known clonal rearrangements followed by testing on previously sequenced clinical samples.

When used in combination with the provided *TCRG* Algorithm worksheet, the assay was capable of detecting DNA from 6 control cell lines (200 ng/µL) diluted into polyclonal tonsil DNA (200 ng/µL) at 5% (v/v).

Furthermore, the performance of the *TCRG* 2.0 Assay was evaluated on clinical samples for which the T-cell receptor gamma gene rearrangement status had been identified by Roche 454 sequencing. For the 7 samples that had been identified as clonal by sequencing, the *TCRG* 2.0 assay had 100% concordance. For the 12 samples that were either negative for a clonal event or were oligoclonal, concordance of the *TCRG* 2.0 assay was 75%. Sample types included peripheral blood, bone marrow, and formalin-fixed, paraffin embedded (FFPE) tissue.

Always interpret the results of molecular clonality tests in the context of clinical, histological and immunophenotypic data.

## Reference

1. Miller JE, Wilson SS, Jaye DJ, and Kronenberg M. *Mol. Diag.* 1999, 4(2):101-117.

2. Armand, Marine et al. *HemaSphere*, 2019;3:3.

EuroClonality

**This assay was developed by Invivoscribe.**
**The performance of this assay was reviewed and validated by the EuroClonality/BIOMED-2 Group.**[2]

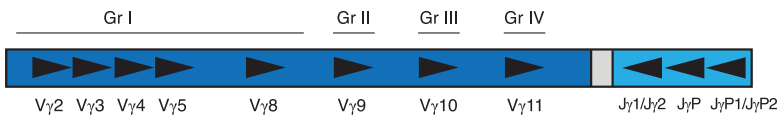



**Figure Legend:** Simple representation of the organization of the T-cell receptor gamma gene on chromosome 7p14. Black arrows represent the relative positions of primers that target the variable region genes and the downstream joining region gene segments that are involved in rearrangements in T-cell lymphomas. The downstream primers are fluorescently labeled through the incorporation of a 6FAM fluorophore. The amplicon products generated from these rearrangements are detected by capillary electrophoresis.

# IdentiClone®

T-Cell Receptor Gamma Gene Rearrangement Assay 2.0

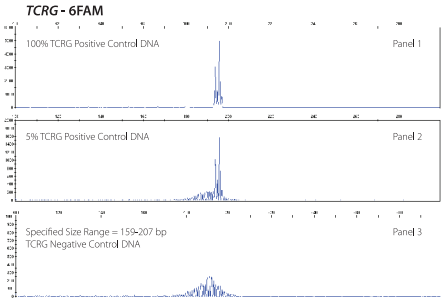
Gel and Capillary CE IVD Assays

## Reagents

| Controls | Concentration | Units in Assay | Units in Assay MegaKit |
|---|---|---|---|
| 5% *TCRG* Positive Control DNA | 50 µg/mL | 1 x 50 µL tube | 5 x 50 µL tube |
| *TCRG* Negative Control DNA | 50 µg/mL | 1 x 50 µL tube | 5 x 50 µL tube |
| **Master Mixes** | **Target** | **Units in Assay** | **Units in Assay MegaKit** |
| *TCRG* - 6FAM | Vγ1-Vγ11 + Jγ1/Jγ2, JγP, JγP1/JγP2 | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| Specimen Control Size Ladder | Multiple Genes | 1 x 1500 µL tube | 10 x 1500 µL tubes |

## Capillary Electrophoresis Detection (ABI)

Fluorescence detection is commonly used to resolve the different sized amplicon products using a capillary electrophoresis instrument. Primers are conjugated with a 6-FAM fluorescent dye (fluorophore), so that they can be detected upon excitation by laser. This detection system results in a high sensitivity, single nucleotide size resolution, and relative quantification. Inter- and intra-assay reproducibility in size determination using capillary electrophoresis is approximately 1 to 4 nucleotides. The data shown were generated using the *TCRG*–6FAM master mix. Amplified products were run on an ABI 3130 instrument.




## Ordering Information

| Catalog # | Products | Quantity |
|---|---|---|
| 9-207-0101 | IdentiClone® T-Cell Receptor Gamma Gene Rearrangement Assay 2.0 - ABI Fluorescence Detection | 33 reactions |
| 9-207-0111 | IdentiClone® T-Cell Receptor Gamma Gene Rearrangement Assay MegaKit 2.0 - ABI Fluorescence Detection | 330 reactions |

These are *in vitro* diagnostic products, and are not available for sale or use within North America.

# *TCRG* Gene Clonality Assays

## Assay Description

The IdentiClone *TCRG* Gene Clonality Assays are *in vitro* diagnostic products intended for PCR-based detection of clonal T-cell receptor gamma chain gene rearrangements in patients with suspect lymphoproliferations.

Specifically, the IdentiClone *TCRG* Gene Clonality Assays can be used to:

- Identify clonality in suspect lymphoproliferations
- Support a differential diagnosis between reactive lesions and T-cell and some immature B-cell malignancies
- Determine lineage involvement in mature lymphoproliferative disorders
- Monitor and evaluate disease recurrence

## Summary and Explanation of the Test

The Invivoscribe CE-marked IdentiClone Assays represent a unique approach to PCR-based clonality testing. These standardized assays were carefully optimized testing positive and negative control samples using multiplex master mixes. Assay development was followed by extensive validation including the testing of more than 400 clinical samples using Revised European/American Lymphoma (REAL) Classification. Testing was done at more than thirty prominent independent testing centers throughout Europe in a collaborative study known as the BIOMED-2 Concerted Action. Results from this BIOMED-2 study appear in *Leukemia*, a leading peer-reviewed journal.[1]

These test kits include three master mixes targeting *TRG* (formerly known as *TCRG*) gene rearrangements. *TCRG* Tube A contains primers that target the Vγ1-8 + Vγ10 genes and Jγ1.1, Jγ1.3, Jγ2.1, and Jγ2.3 genes (also known as JγP1, Jγ1, JγP2, and Jγ2 respectively). *TCRG* Tube B contains primers that target the Vγ9 + Vγ11 genes and Jγ1.1, Jγ1.3, Jγ2.1, and Jγ2.3 genes. The Specimen Control Size Ladder master mix targets multiple genes and generates a series of amplicons of approximately 100, 200, 300, 400, and 600 base pairs to ensure that the quality and quantity of input DNA is adequate to yield a valid result. A single thermal cycler program and similar detection methodologies are used with all of our gene clonality assays. This improves consistency and facilitates cross-training.

## Performance Characteristics

Data from an independent, peer-reviewed study suggests the diagnostic accuracy of selected IdentiClone tests to be 96%. There were no clear false-positive results generated using the IdentiClone tests, and there was a high level of precision. The clinico-histopathological diagnosis correlates well with PCR results in a higher number of patients when compared with Southern Blot (SB) results, as seen below:

| | PCR/SB concordance:[1] |
|---|---|
| *IGH:* | 93% sensitivity / 92% specificity |
| *IGK:* | 90% sensitivity / 90% specificity |
| *IGL:* | 86% sensitivity / 92% specificity |
| *TRB:* | 86% sensitivity / 98% specificity |
| *TRG:* | 89% sensitivity / 94% specificity |
| *TRD:* | 83% sensitivity / 95% specificity |

## Reference

1. JJM van Dongen et al., *Leukemia* 17:2257-2317 (2003).

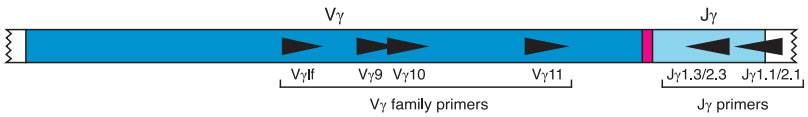



**Figure Legend:** Simple representation of the organization of the T-cell receptor gamma chain gene on chromosome 7. Black arrows represent the relative positions of primers that target the variable (V*γ*) regions, and the downstream joining (J*γ*) gene segments. The amplicon products generated from each of these regions can be differentially detected when fluorescent primer sets are used with capillary electrophoresis instruments that employ differential fluorescence detection.

*TCRG* tube A: Vγlf and Vγ10 primers + Jγ1.1/2.1 and Jγ1.3/2.3

*TCRG* tube B: Vγ9 and Vγ11 primers + Jγ1.1/2.1 and Jγ1.3/2.3

EuroClonality This assay is based on the **EuroClonality/BIOMED-2** Concerted Action BMH4-CT98-3936.

# IdentiClone®

*TCRG* Gene Clonality Assays

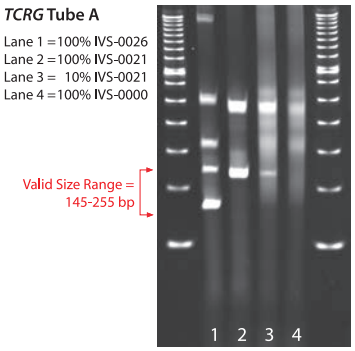
Gel and Capillary CE IVD Assays

## Reagents

| Controls | Concentration | Units in Assay | Units in Assay MegaKit |
|---|---|---|---|
| IVS-0009 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0021 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0000 Polyclonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| **Master Mixes** | **Target** | **Units in Assay** | **Units in Assay MegaKit** |
| *TCRG* Tube A | Vγ1-8 + Vγ10 + Jγ | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *TCRG* Tube B | Vγ9 + Vγ11 + Jγ | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| Specimen Control Size Ladder | Multiple Genes | 1 x 1500 µL tube | 10 x 1500 µL tubes |

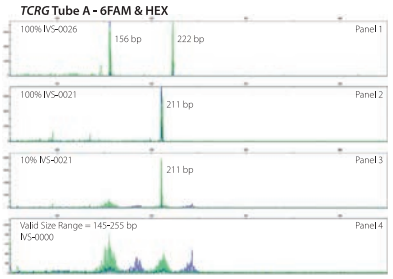
## Gel Electrophoresis Detection

Heteroduplex analysis is performed to differentiate clonal and non-clonal PCR products. It involves heat denaturation of double-stranded DNA, followed by a snap chilling process to force DNA strands to quickly reanneal. In non-clonal populations, this process causes a majority of the single-stranded DNA to incorrectly bind to non-homologous strands, resulting in secondary structures and reducing the DNA's ability to migrate through a non-denaturing polyacrylamide gel. As a result, polyclonal products are frequently observed as smears at high molecular weights. In clonal populations, after heteroduplex analysis is performed, most denatured single-strand PCR products will reanneal correctly with homologous strands of DNA (reforming homoduplexes), allowing them to easily migrate through the polyacrylamide gel as a single band. Therefore, heteroduplex analysis is key for analyzing PCR products visualized using gel detection methods, as it increases the separation between clonal and polyclonal products.




## Capillary Electrophoresis Detection (ABI)

Differential fluorescence detection, such as ABI Fluorescence Detection, is commonly used to resolve different-sized amplicon products using a capillary electrophoresis instrument. Primers can be conjugated with several different fluorescent dyes (fluorophores), so that they produce different emission spectra upon excitation by a laser in the capillary electrophoresis instrument. In this manner, different fluorescent dyes can correspond to different targeted regions. This detection system results in excellent sensitivity, single nucleotide resolution, differential product detection, and relative quantification. In addition, differential detection allows accurate, reproducible and objective interpretation of primer-specific products. Inter-assay and intra-assay reproducibility in size determination using capillary electrophoresis is approximately 1 to 4 nucleotides.




## Ordering Information

| Catalog # | Products | Quantity |
|---|---|---|
| 9-207-0020 | IdentiClone® *TCRG* Gene Clonality Assay - Gel Detection | 33 reactions |
| 9-207-0040 | IdentiClone® *TCRG* Gene Clonality Assay MegaKit - Gel Detection | 330 reactions |
| 9-207-0021 | IdentiClone® *TCRG* Gene Clonality Assay - ABI Fluorescence Detection | 33 reactions |
| 9-207-0041 | IdentiClone® *TCRG* Gene Clonality Assay MegaKit - ABI Fluorescence Detection | 330 reactions |

These are *in vitro* diagnostic products, and are not available for sale or use within North America.

# *TCRD* Gene Clonality Assays

## Assay Description

The IdentiClone *TCRD* Gene Clonality Assays are *in vitro* diagnostic products intended for PCR-based detection of clonal T-cell receptor delta chain gene rearrangements in patients with suspect lymphoproliferations.

Specifically, the IdentiClone *TCRD* Gene Clonality Assays can be used to:

- Identify clonality in suspect lymphoproliferations
- Support a differential diagnosis between reactive lesions and T-cell and some immature B-cell malignancies
- Determine lineage involvement in mature lymphoproliferative disorders
- Monitor and evaluate disease recurrence

## Summary and Explanation of the Test

The Invivoscribe CE-marked IdentiClone Assays represent a unique approach to PCR-based clonality testing. These standardized assays were carefully optimized testing positive and negative control samples using multiplex master mixes. Assay development was followed by extensive validation including the testing of more than 400 clinical samples using Revised European/American Lymphoma (REAL) Classification. Testing was done at more than thirty prominent independent testing centers throughout Europe in a collaborative study known as the BIOMED-2 Concerted Action. Results from this BIOMED-2 study appear in *Leukemia*, a leading peer-reviewed journal.[1]

These test kits include two master mixes. The *TCRD* tube targets the framework regions within the variable region, the diversity region, and the joining region of the T-cell receptor delta chain locus (*TRD*, formerly known as *TCRD*). The Specimen Control Size Ladder master mix targets multiple genes and generates a series of amplicons of approximately 100, 200, 300, 400, and 600 base pairs to ensure that the quality and quantity of input DNA is adequate to yield a valid result. A single thermal cycler program and similar detection methodologies are used with all of our gene clonality assays. This improves consistency and facilitates cross-training.

**Figure Legend:** Simplified diagram of a representative rearranged T-cell receptor delta gene showing the approximate placement of the upstream and downstream DNA primers. The numbers of primers and their specificity are listed for the *TRD* Tube Master Mix tube.

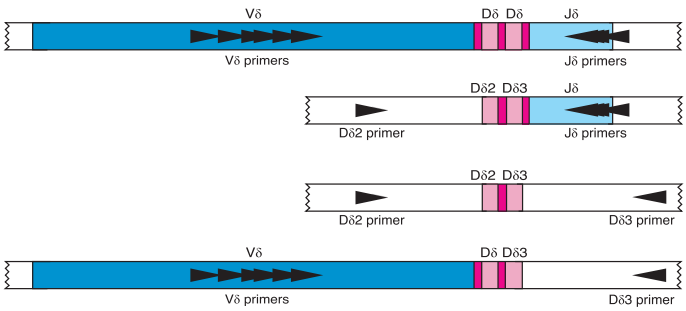
## Performance Characteristics

Data from an independent, peer-reviewed study suggests the diagnostic accuracy of selected IdentiClone tests to be 96%. There were no clear false-positive results generated using the IdentiClone tests, and there was a high level of precision. The clinico-histopathological diagnosis correlates well with PCR results in a higher number of patients when compared with Southern Blot (SB) results, as seen below:

| | PCR/SB concordance:[1] |
|---|---|
| *IGH:* | 93% sensitivity / 92% specificity |
| *IGK:* | 90% sensitivity / 90% specificity |
| *IGL:* | 86% sensitivity / 92% specificity |
| *TRB:* | 86% sensitivity / 98% specificity |
| *TRG:* | 89% sensitivity / 94% specificity |
| *TRD:* | 83% sensitivity / 95% specificity |

## Reference

1. JJM van Dongen et al., *Leukemia* 17:2257-2317 (2003).




*TCRD* tube: 6 Vδ and 1 Dδ2 primers + 4 Jδ and 1 Dδ3 primers

EuroClonality This assay is based on the **EuroClonality/BIOMED-2** Concerted Action BMH4-CT98-3936.

# IdentiClone®

*TCRD* Gene Clonality Assays

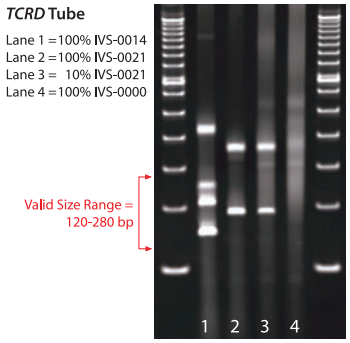
Gel and Capillary CE IVD Assays

## Reagents

| Controls | Concentration | Units in Assay | Units in Assay MegaKit |
|---|---|---|---|
| IVS-0021 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0000 Polyclonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| **Master Mixes** | **Target** | **Units in Assay** | **Units in Assay MegaKit** |
| *TCRD* Tube | Multiple Vδ + Dδ +Jδ | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| Specimen Control Size Ladder | Multiple Genes | 1 x 1500 µL tube | 10 x 1500 µL tubes |

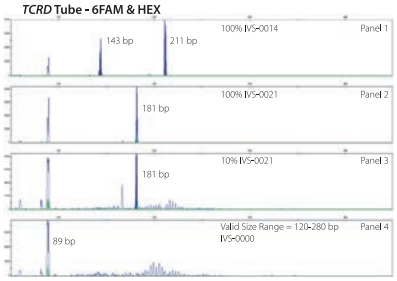
## Gel Electrophoresis Detection

Heteroduplex analysis is performed to differentiate clonal and non-clonal PCR products. It involves heat denaturation of double-stranded DNA, followed by a snap chilling process to force DNA strands to quickly reanneal. In non-clonal populations, this process causes a majority of the single-stranded DNA to incorrectly bind to non-homologous strands, resulting in secondary structures and reducing the DNA's ability to migrate through a non-denaturing polyacrylamide gel. As a result, polyclonal products are frequently observed as smears at high molecular weights. In clonal populations, after heteroduplex analysis is performed, most denatured single-strand PCR products will reanneal correctly with homologous strands of DNA (reforming homoduplexes), allowing them to easily migrate through the polyacrylamide gel as a single band. Therefore, heteroduplex analysis is key for analyzing PCR products visualized using gel detection methods, as it increases the separation between clonal and polyclonal products.




## Capillary Electrophoresis Detection (ABI)

Differential fluorescence detection, such as ABI Fluorescence Detection, is commonly used to resolve different-sized amplicon products using a capillary electrophoresis instrument. Primers can be conjugated with several different fluorescent dyes (fluorophores), so that they produce different emission spectra upon excitation by a laser in the capillary electrophoresis instrument. In this manner, different fluorescent dyes can correspond to different targeted regions. This detection system results in excellent sensitivity, single nucleotide resolution, differential product detection, and relative quantification. In addition, differential detection allows accurate, reproducible and objective interpretation of primer-specific products. Inter-assay and intra-assay reproducibility in size determination using capillary electrophoresis is approximately 1 to 4 nucleotides.




## Ordering Information

| Catalog # | Products | Quantity |
|---|---|---|
| 9-206-0010 | IdentiClone® *TCRD* Gene Clonality Assay - Gel Detection | 33 reactions |
| 9-206-0020 | IdentiClone® *TCRD* Gene Clonality Assay MegaKit - Gel Detection | 330 reactions |
| 9-206-0011 | IdentiClone® *TCRD* Gene Clonality Assay - ABI Fluorescence Detection | 33 reactions |
| 9-206-0021 | IdentiClone® *TCRD* Gene Clonality Assay MegaKit - ABI Fluorescence Detection | 330 reactions |

These are *in vitro* diagnostic products, and are not available for sale or use within North America.

# *BCL1*/JH Translocation Assay

## Assay Description

The IdentiClone *BCL1*/JH Translocation Assay is an *in vitro* diagnostic product intended for PCR-based detection of *BCL1*/JH t(11;14)(q13;q32) gene translocations in patients with suspect lymphoproliferations.

Specifically, the *BCL1*/JH Translocation Assay can be used to:

- Identify *BCL1*/JH gene translocations highly suggestive of mantle cell lymphoma (MCL)
- Distinguish mantle cell lymphoma from other neoplastic or benign B-cell proliferations
- Monitor and evaluate disease recurrence

## Summary and Explanation of the Test

The Invivoscribe CE-marked IdentiClone assays represent a unique approach to PCR-based clonality testing. These standardized assays were carefully optimized testing positive and negative control samples using multiplex master mixes. Assay development was followed by extensive validation including the testing of more than 400 clinical samples using Revised European/American Lymphoma (REAL) Classification. Testing was done at more than thirty prominent independent testing centers throughout Europe in a collaborative study known as the BIOMED-2 Concerted Action. Results from this BIOMED-2 study appear in *Leukemia*, a leading peer-reviewed journal.[1]

These test kits include includes two master mixes. The *BCL1*/JH Tube targets the major translocation cluster (MTC) of the *IGH-CCND1* locus and the joining region of the immunoglobulin heavy chain locus. The Specimen Control Size Ladder master mix targets multiple genes and generates a series of amplicons of approximately 100, 200, 300, 400, and 600 base pairs to ensure that the quality and quantity of input DNA is adequate to yield a valid result. A single thermal cycler program and similar detection methodologies are used with many of our assays. This improves consistency and facilitates cross-training.

## Performance Characteristics

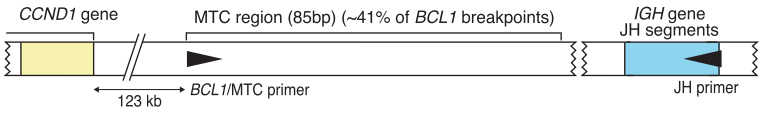
The assay analytical performance was evaluated by testing spiked Mantle Cell Lymphoma (MCL) *IGH-CCND1* positive cell-line DNA into tonsil DNA at six different dilutions. The Limit of Detection (LoD) was observed at 0.1% DNA dilution. To evaluate within-laboratory precision, complete agreement of results was observed across four runs executed by two operators over two days.

Testing conducted across three laboratories using 25 samples from cases of MCL with *IGH-CCND1* translocations and 18 negative samples, showed 100% concordance of positive samples (25 of 25 samples) using fluorescence detection, and 88% (22 of 25 samples) using gel detection. For the negative samples, the concordance was 100% using both gel detection (18 of 18 samples) and fluorescence detection (18 of 18 samples) formats. Specificity for both formats was 100% and sensitivity was determined to be between $10^{-3}$ and $10^{-4}$. The sensitivity is sufficiently high for the detection of the *IGH-CCND1* breakpoint in diagnostic material. However, only 40-50% of the t(11;14) breakpoints in MCL will be detected by PCR alone and additional detection method tools are recommended for diagnosis of breakpoints that do not fall within the major translocation cluster region.

## Reference

1. JJM van Dongen et al., *Leukemia* 17:2257-2317 (2003).

**Figure Legend:** Schematic diagram of the *IGH-CCND1* t(11;14) translocation showing the cyclin D1 (*CCND1*) gene on the left and the Ig heavy chain (*IGH*) gene on the right. Shown are the relative positions and orientations for the *BCL1*/MTC primer and the JH primer, which are included in the *BCL1*/JH Master Mix tube.




t(11;14) tube: 1 *BCL1* MTC primer + 1 JH primer

EuroClonality

This assay is based on the **EuroClonality/BIOMED-2** Concerted Action BMH4-CT98-3936.

# IdentiClone®

*BCL1*/JH Translocation Assay

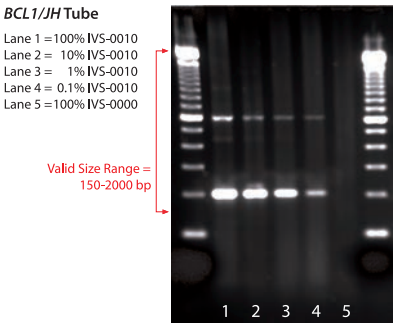
Gel and Capillary CE IVD Assays

## Reagents

| Controls | Concentration | Units in Assay | Units in Assay MegaKit |
|---|---|---|---|
| IVS-0010 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0000 Polyclonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| **Master Mixes** | **Target** | **Units in Assay** | **Units in Assay MegaKit** |
| *BCL1*/JH Tube - Unlabeled | MTC of *BCL1* + *IGH* JH | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| Specimen Control Size Ladder - Unlabeled | Multiple Genes | 1 x 1500 µL tube | 10 x 1500 µL tubes |

## Gel Electrophoresis Detection

Gel electrophoresis, such as agarose gel electrophoresis, is commonly used to resolve different amplicon products based on their size, charge, and conformation. Since DNA is negatively charged, when an electrical potential (voltage) is applied across the gel containing PCR products, the electrical field causes the amplicons to migrate through the gel. Smaller DNA fragments are able to easily migrate through the gel matrix, whereas larger DNA fragments migrate more slowly. This causes a separation of the amplicon products based on size. Ethidium bromide or other DNA intercalating dyes can then be used to stain and detect these products in the gel. The use of a DNA ladder allows the relative size of amplicons to be determined.




## Ordering Information

| Catalog # | Products | Quantity |
|---|---|---|
| 9-308-0010 | IdentiClone® *BCL1*/JH Translocation Assay - Gel Detection | 33 reactions |
| 9-308-0020 | IdentiClone® *BCL1*/JH Translocation Assay MegaKit - Gel Detection | 330 reactions |

These are *in vitro* diagnostic products, and are not available for sale or use within North America.

# *BCL2*/JH Translocation Assay

## Assay Description

The IdentiClone *BCL2*/JH Translocation Assay is an *in vitro* diagnostic product intended for PCR-based detection of *IGH-BCL2* t(14;18) gene translocations in patients with suspect lymphoproliferations.

Specifically, the *BCL2*/JH Translocation Assay can be used to:

- Distinguish lymphoma from benign lymphoid hyperplasia
- Distinguish follicular lymphoma (FL) from other B-cell lymphomas that may have a similar appearance
- Monitor and evaluate disease recurrence

## Summary and Explanation of the Test

The Invivoscribe CE-marked IdentiClone Assays represent a unique approach to PCR-based clonality testing. These standardized assays were carefully optimized testing positive and negative control samples using multiplex master mixes. Assay development was followed by extensive validation including the testing of more than 400 clinical samples using Revised European/American Lymphoma (REAL) Classification. Testing was done at more than thirty prominent independent testing centers throughout Europe in a collaborative study known as the BIOMED-2 Concerted Action. Results from this BIOMED-2 study appear in *Leukemia*, a leading peer-reviewed journal.[1]

These test kits include four master mixes. The *BCL2*/JH Translocation master mixes (*BCL2*/JH Tubes A, B, and C) target the joining (J) region of the immunoglobulin heavy chain (*IGH*) gene and distinct regions of the *BCL2* gene. These master mixes are used to detect major breakpoint region (MBR) and minor cluster region (mcr) of the *IGH-BCL2* t(14;18)(q32;q21) translocations. The Specimen Control Size Ladder master mix targets multiple genes and generates a series of amplicons of approximately 100, 200, 300, 400, and 600 base pairs to ensure that the quality and quantity of input DNA is adequate to yield a valid result. A single thermal cycler program and similar detection methodologies are used with many of our assays. This improves consistency and facilitates cross-training.

## Performance Characteristics

The initial evaluation of this assay was performed in three laboratories on DNA derived from 124 cases of follicular cell lymphoma (FCL) known to carry the t(14;18) translocation. 109 cases were identified with the *IGH-BCL2* fusion gene (88%) using this PCR assay. The final testing and evaluation was done on samples in 11 independent laboratories[1]. False-positive results (0.4%) were only seen in 12 of 3036 analyses.

This IdentiClone *BCL2*/JH Translocation Assay was found to be more sensitive than Southern blot analysis. Sensitivity differed slightly between the master mixes. However, overall sensitivity for the assay was determined to be between 1 positive cell in $10^2$ normal cells and 1 positive cell in $10^3$ normal cells.

In conclusion, we have designed and evaluated the performance characteristics of a robust three tube multiplex PCR assay in order to maximize the detection of the t(14;18) breakpoint. This strategy is capable of amplifying across the breakpoint region in the majority of cases of follicular lymphoma with a cytogenetically defined translocation.

## Reference

1. JJM van Dongen et al., *Leukemia* 17:2257 - 2317 (2003).


**Figure Legend:** Schematic diagram of the *IGH-BCL2* t(14;18) translocation showing the *BCL2* gene on the left and the Ig heavy chain (*IGH*) gene on the right. Shown are the relative positions and orientations for the major breakpoint region (MBR) primers, the minor cluster region (mcr) primers, and the JH primer, which are included in the 3 *BCL2*/JH master mix tubes.

t(14;18) tube A: 2 *BCL2* MBR primers + 1 JH primer

t(14;18) tube B: 4 *BCL2* 3'MBR primers + 1 JH primer

t(14;18) tube C: 3 *BCL2* mcr primers + 1 JH primer

EuroClonality This assay is based on the **EuroClonality/BIOMED-2** Concerted Action BMH4-CT98-3936.

# IdentiClone®

*BCL2*/JH Translocation Assay

Gel and Capillary CE IVD Assays

## Reagents

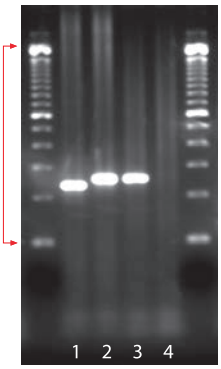
| Controls | Concentration | Units in Assay | Units in Assay MegaKit |
|---|---|---|---|
| IVS-0030 Clonal Control DNA | 100 µL @ 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-P002 Clonal Control DNA | 100 µL @ 1600 pg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0031 Clonal Control DNA | 100 µL @ 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0000 Polyclonal Control DNA | 100 µL @ 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| **Master Mixes** | **Target** | **Units in Assay** | **Units in Assay MegaKit** |
| *BCL2*/JH Tube A - Unlabeled | *BCL2* MBR + *IGH* JH | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *BCL2*/JH Tube B - Unlabeled | *BCL2* 3′ MBR + *IGH* JH | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *BCL2*/JH Tube C - Unlabeled | *BCL2* mcr + *IGH* JH | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| Specimen Control Size Ladder - Unlabeled | Multiple Genes | 1 x 1500 µL tube | 10 x 1500 µL tubes |

## Gel Electrophoresis Detection

Gel electrophoresis, such as agarose gel electrophoresis, is commonly used to resolve different amplicon products based on their size, charge, and conformation. Since DNA is negatively charged, when an electrical potential (voltage) is applied across the gel containing PCR products, the electrical field causes the amplicons to migrate through the gel. Smaller DNA fragments are able to easily migrate through the gel matrix, whereas larger DNA fragments migrate more slowly. This causes a separation of the amplicon products based on size. Ethidium bromide or other DNA intercalating dyes can then be used to stain and detect these products in the gel.

***BCL2/JH* Tube A**

Lane 1 = 100% IVS-0007
Lane 2 = 100% IVS-0030
Lane 3 = 1% IVS-0030
Lane 4 = 100% IVS-0000




## Ordering Information

| Catalog # | Products | Quantity |
|---|---|---|
| 9-309-0020 | IdentiClone® *BCL2*/JH Translocation Assay - Gel Detection | 33 reactions |
| 9-309-0040 | IdentiClone® *BCL2*/JH Translocation Assay MegaKit - Gel Detection | 330 reactions |

These are *in vitro* diagnostic products, and are not available for sale or use within North America.

# LeukoStrat®

## Gel and Capillary CE IVD Assays and IVD Assays

# LeukoStrat®

## LeukoStrat Assay kits are *in vitro* diagnostic products.*

These assay kits are intended for PCR-based detection of *FLT3* activating mutations in patients with acute myelogenous leukemia (AML) using gel or capillary electrophoresis methods.

These tests include Instructions For Use (IFUs) with interpretation guidelines describing the use of the kits' master mixes and controls. Master mixes are composed of a buffered magnesium chloride solution, deoxynucleotides, and primers targeting the gene segments of interest. These assay master mixes are complete other than Taq DNA polymerase.

In the Japan marketed IVD product, Tag DNA Polymerase and EcoRV Enzyme are further included in the LeukoStrat Assay Kit.

These assays are available in regular (33 reactions) and in MegaKit formats (330 reactions), with exception of the LeukoStrat® CDx *FLT3* Mutation Assay, which is only available in regular format.

For more information, please visit www.invivoscribe.com

*NOTICE: LeukoStrat Assays are *in vitro* diagnostic products and are not available for sale or use within North America. For more information regarding the research use only reagents, please see the Gel & Capillary Research Use Only Assays section.

# *FLT3* Mutation Assay - Gel Detection

## Assay Description

The LeukoStrat® *FLT3* Mutation Assay is an *in vitro* diagnostic product intended for PCR-based detection of *FLT3* activating mutations in patients with acute myelogenous leukemia (AML).

Specifically, the *FLT3* Mutation Assay can be used to:

- Identify internal tandem duplications (ITD) in the *FLT3* gene
- Identify tyrosine kinase domain (TKD) mutations in the *FLT3* gene

## Summary and Explanation of the Test

AML in general has a poor prognosis. Assessment of the mutation status of the *FLT3* (fms related tyrosine kinase 3) receptor gene in karyotype normal AML is the most important prognostic indicator of disease outcome, which is often substantial, as many studies in AML have shown that the presence of *FLT3* activating mutations portends a poor prognosis.[1,2] For this reason, *FLT3* activation mutation testing is required to stratify disease and determine appropriate treatment options. This LeukoStrat PCR assay targets regions of the *FLT3* gene to identify ITD mutations and TKD mutations, such as the D835 and I836 mutations.

This assay cannot reliably detect *FLT3* mutations comprising less than 5% of the total cell population. It should be emphasized that the results of molecular mutation tests should always be interpreted in the context of clinical, histological and immunophenotypic data.

This test kit includes 3 master mixes. (1) *FLT3* ITD Master mix tests for internal tandem duplication mutations. (2) *FLT3* D835 Master Mix tests for TKD region mutations. (3) The Specimen Control Size Ladder master mix targets multiple genes and generates a series of amplicons of approximately 100, 200, 300, 400, and 600 basepairs to ensure that the quality and quantity of input DNA is adequate to yield a valid result.

## Performance Characteristics

This LeukoStrat *FLT3* Mutation Assay offers a rapid and reliable method for detecting *FLT3* mutations. This is shown by a validation performed by the Laboratory for Personalized Molecular Medicine (LabPMM).

| | Concordance with three independent labs |
|---|---|
| ITD | 100% sensitivity / 100% specificity |
| TKD | 100% sensitivity / 100% specificity |

LabPMM tested 57 blinded patient samples obtained from three independent institutions. The institutions determined that 13 of the samples were *FLT3* ITD positive, 33 were *FLT3* ITD negative, 6 were *FLT3* TKD positive, and 50 were *FLT3* TKD negative. In addition 10 positive blinded spiked samples and 10 negative samples were used for the validation of *FLT3* TKD. The LeukoStrat *FLT3* Mutation Assay showed a sensitivity and specificity of 100% with both master mixes. The analytical sensitivity of both master mixes was determined to be 5 positive cells out of 100 total cells.

## Reference

1. Murphy KM et al., A Clinical PCR/Capillary Electrophoresis Assay for the Detection of Internal Tandem Duplication and Point Mutation of the *FLT3* Gene. *J. Mol. Diag.* 5:96-102 (2003).
2. Yamamoto, Y., et al., Activating mutation of D835 within the activation loop of *FLT3* in human hematologic malignancies. *Blood*, 97(8):2434-9 (2001).

# LeukoStrat®

*FLT3* Mutation Assay - Gel Detection

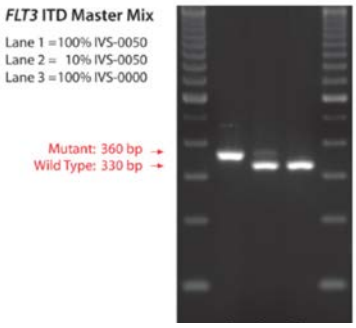
Gel and Capillary CE IVD Assays and IVD Assays

## Reagents

| Controls | Concentration | Units in 33 Reaction Assay | Units in 330 Reaction Assay |
|---|---|---|---|
| IVS-0050 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-P004 Clonal Control DNA | 171 pg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0000 Polyclonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| **Master Mixes** | **Target** | **Units in 33 Reaction Assay** | **Units in 330 Assay MegaKit** |
| *FLT3* ITD Master Mix - Unlabeled | *FLT3* ITD | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *FLT3* ITD Master Mix - Unlabeled | *FLT3* TKD | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| Specimen Control Size Ladder | Multiple Genes | 1 x 1500 µL tube | 10 x 1500 µL tubes |

## Gel Electrophoresis Detection

Agarose gel electrophoresis is used to resolve the different amplicon products based on their size, charge, and conformation. Since DNA is negatively charged, when an electrical potential (voltage) is applied across the gel containing PCR products, the electrical field causes the amplicons to migrate through the gel. Smaller DNA fragments are able to easily migrate through the gel matrix, whereas larger DNA fragments migrate more slowly. This causes a separation of the amplicon products based on size. Ethidium bromide or other DNA intercalating dyes can then be used to stain and detect these products in the gel.




## Ordering Information

| Catalog # | Products | Quantity |
|---|---|---|
| 9-412-0010 | LeukoStrat® *FLT3* Mutation Assay - Gel Detection | 33 reactions |
| 9-412-0020 | LeukoStrat® *FLT3* Mutation Assay MegaKit - Gel Detection | 330 reactions |

These are *in vitro* diagnostic products, and are not available for sale or use within North America.

# *FLT3* Mutation Assay 2.0 - ABI Fluorescence Detection

## Assay Description

The LeukoStrat® *FLT3* Mutation Assay 2.0 is an *in vitro* diagnostic product intended for PCR-based detection of *FLT3* activating mutations in patients with acute myelogenous leukemia (AML).

Specifically, the *FLT3* Mutation Assay 2.0 can be used to:

- Identify internal tandem duplications (ITD) in the *FLT3* gene
- Identify tyrosine kinase domain (TKD) mutations in the *FLT3* gene

## Summary and Explanation of the Test

AML in general has a poor prognosis.[1,2] Assessment of the mutation status of the *FLT3* (fms related tyrosine kinase 3) receptor gene is the most important prognostic indicator of disease outcome, occurring in approximately 30% of patients at the time of diagnosis.[3] For this reason, testing for *FLT3* activating mutations is required for the stratification of disease and determination of appropriate treatment options. The LeukoStrat *FLT3* Mutation Assay 2.0 is a PCR-based method that identifies ITD and TKD mutations. This test kit includes 2 master mixes: the *FLT3*-ITD Master Mix for the detection of ITD mutations and *FLT3*-D835 Master Mix for the detection of TKD region mutations (such as the D835 and I836 mutations).

## Performance Characteristics

The LeukoStrat *FLT3* Mutation Assay 2.0 is a rapid and reliable method for the detection of *FLT3* mutations, as evidenced by comparison with Roche® 454 sequencing.

The *FLT3* Mutation Assay 2.0 is capable of detecting *FLT3*-ITD and TKD mutations with excellent concordance (Table 1 and 2) and has high reliability when multiple standard laboratory variables are considered, including multiple operators, reagent lots, different ABI 3500xL instruments, and nonconsecutive testing days.

Table 1. *FLT3* ITD Percent Agreement with 454 Sequencing

| Percent Agreement | | Discordance # | Concordance # | *95% LL |
|---|---|---|---|---|
| Negative PA | 100% | 0 | 119 | 96.9% |
| Positive PA | 98.0% | 4 | 200 | 95.1% |

*95% of results would be expected to agree with sequencing at a rate greater than or equal to the lower limit (LL).

Table 2. *FLT3* TKD Percent Agreement with 454 Sequencing

| Percent Agreement | | Discordance # | Concordance # | *95% LL |
|---|---|---|---|---|
| Negative PA | 100% | 0 | 137 | 96.9% |
| Positive PA | 100% | 0 | 240 | 98.5% |

*95% of results would be expected to agree with sequencing at a rate greater than or equal to the lower limit (LL).

## Reference

1. Murphy KM et al., A Clinical PCR/Capillary Electrophoresis Assay for the Detection of Internal Tandem Duplication and Point Mutation of the *FLT3* Gene. *J. Mol. Diag.* 5:96-102 (2003).
2. Yamamoto, Y., et al., Activating mutation of D835 within the activation loop of *FLT3* in human hematologic malignancies. *Blood*, 97(8):2434-9 (2001).
3. Acute Myeloid Leukemia, Clinical Practice Guidelines in Oncology, National Comprehensive Cancer Network (v.2.2014)

# LeukoStrat®

*FLT3* Mutation Assay 2.0
ABI Fluorescence Detection

Gel and Capillary CE IVD Assays and IVD Assays

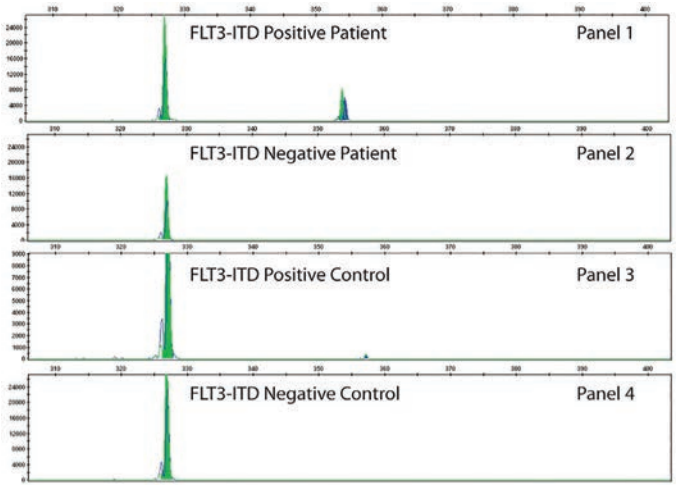
## Reagents

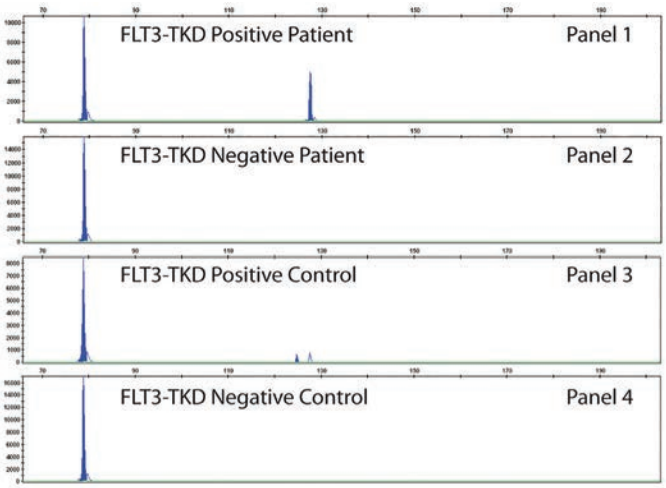
| Controls | Concentration | Units in Assay | Units in MegaKit |
|---|---|---|---|
| *FLT3* ITD Positive Control | 50 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| *FLT3* D835 Positive Control | 50 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| *FLT3* Negative Control | 50 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| **Master Mixes** | **Target** | **Units in Assay** | **Units in MegaKit** |
| *FLT3* ITD Master Mix – 6FAM & HEX | *FLT3* ITD | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *FLT3* D835 Master Mix – 6FAM | *FLT3* TKD | 1 x 1500 µL tube | 10 x 1500 µL tubes |

## Capillary Electrophoresis Detection (ABI)

Differential fluorescence detection, such as ABI fluorescence detection, is commonly used to resolve different-sized amplicon products using a capillary electrophoresis instrument. Primers can be conjugated with different fluorescent dyes (fluorophores), so that they produce different emission spectra upon excitation by a laser in the capillary electrophoresis instrument. In this manner, different fluorescent dyes can correspond to different targeted regions. This detection system results in high sensitivity, single nucleotide resolution, differential product detection, and relative quantification. In addition, differential detection allows accurate, reproducible and objective interpretation of primer-specific products. Inter-assay and intra-assay reproducibility in size determination using capillary electrophoresis is approximately 1 to 4 nucleotides.

The data shown was generated using the master mixes indicated. Amplified products were run on an ABI 3500xL instrument.




## Ordering Information

| Catalog # | Products | Quantity |
|---|---|---|
| 9-412-0091 | LeukoStrat® *FLT3* Mutation Assay 2.0 – ABI Fluorescence Detection | 33 reactions |
| 9-412-0101 | LeukoStrat® *FLT3* Mutation Assay 2.0 MegaKit – ABI Fluorescence Detection | 330 reactions |

These are *in vitro* diagnostic products, and are not available for sale or use within North America.

# CDx *FLT3* Mutation Assay (CE-marked)

**The only internationally standardized CE-IVD assay for *FLT3* Signal Ratio mutation analysis for assessment of acute myeloid leukemia (AML) patients eligible for treatment with RYDAPT® (midostaurin) or XOSPATA® (gilteritinib fumarate).**

## Intended Use

The LeukoStrat CDx *FLT3* Mutation Assay is a PCR-based *in vitro* diagnostic test designed to detect internal tandem duplications (ITD) and tyrosine kinase domain (TKD) mutations D835 and I836 in the *FLT3* gene in genomic DNA extracted from mononuclear cells obtained from peripheral blood or bone marrow aspirates of patients diagnosed with acute myelogenous leukemia (AML).

In regions where midostaurin is available, the LeukoStrat CDx *FLT3* Mutation Assay is used as an aid in the assessment of patients with AML for whom RYDAPT® (midostaurin) treatment is being considered.

In regions where gilteritinib fumarate is available, the LeukoStrat CDx *FLT3* Mutation Assay is used as an aid in the assessment of patients with AML for whom XOSPATA® (gilteritinib fumarate) treatment is being considered.

## Summary and Explanation of the Test

AML in general has a poor prognosis. Assessment of the mutation status of the *FLT3* (fms related tyrosine kinase 3) receptor gene in karyotype normal AML is the most important prognostic indicator of disease outcome, which is often substantial, as many studies in AML have shown that the presence of *FLT3* activating mutations portends a poor prognosis.[1,2] The LeukoStrat CDx *FLT3* Mutation Assay targets regions of the *FLT3* gene to identify ITD mutations and TKD mutations, such as the D835 and I836 mutations, and has been validated in an international clinical trial.

The LeukoStrat CDx *FLT3* Mutation Assay includes reagents, equipment, software and procedures for isolating mononuclear cells and extracting DNA from patient specimens to determine if *FLT3* mutations are present. DNA is amplified via PCR and the amplicons are detected via capillary electrophoresis. *FLT3* mutation status is determined by the LeukoStrat CDx *FLT3* Software. A *FLT3* ITD and/or TKD mutation is reported as Positive if the mutant:wild-type signal ratio meets or exceeds the clinical cutoff of 0.05.

## Method Description

**ITD Mutations of *FLT3***

The LeukoStrat CDx *FLT3* Mutation Assay uses fluorescently labeled primers that are in the JM region. Wild-type *FLT3* alleles will amplify and produce a product at 327±1 bp as measured by this assay, while alleles that contain ITD mutations will produce a product that exceeds 330±1 bp (please see Figure, right).

**TKD Mutations of *FLT3***

The LeukoStrat CDx *FLT3* Mutation Assay uses primers that lie on either side of the TKD region. The *FLT3* target region is amplified using PCR and then an EcoRV restriction digest is performed. Wild-type alleles of the *FLT3* gene yield digestion products of 79±1 bp whereas mutant alleles yield products of 125±1 bp or 127±1 bp from the original undigested amplicon product of 145±1 bp or 147±1 bp, as measured by this assay (please see Figure, right).

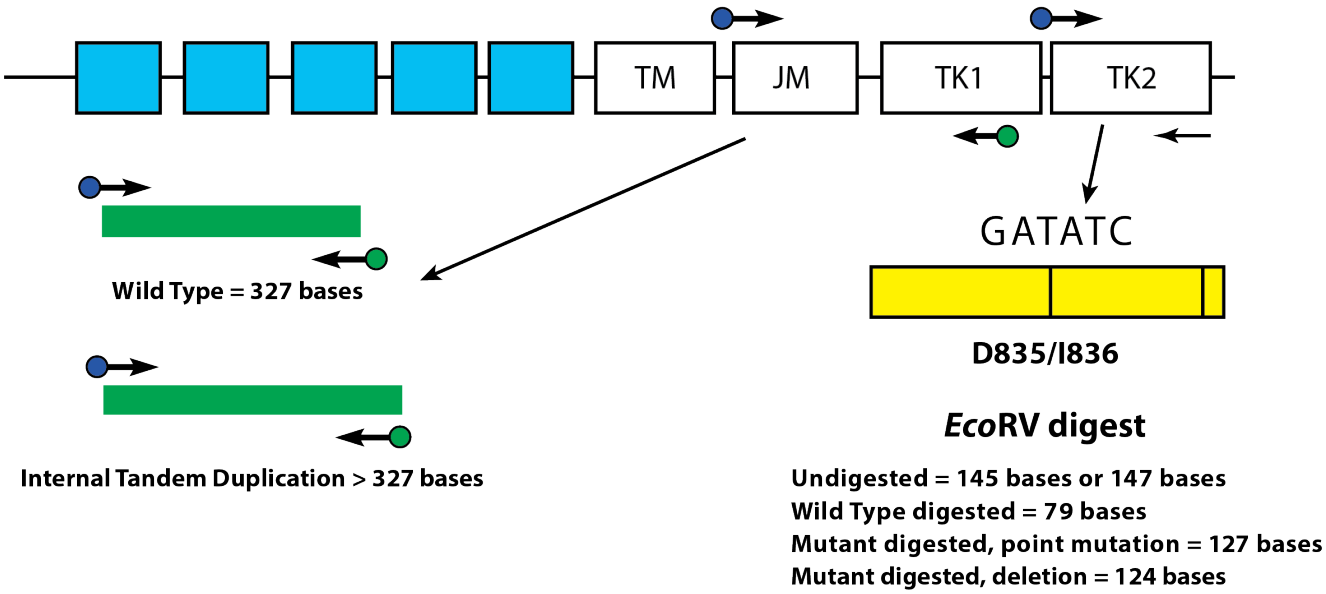
## Reference

1. Murphy KM et al., A Clinical PCR/Capillary Electrophoresis Assay for the Detection of Internal Tandem Duplication and Point Mutation of the *FLT3* Gene. *J. Mol. Diag.* 5:96-102 (2003).
2. Yamamoto, Y., et al., Activating mutation of D835 within the activation loop of *FLT3* in human hematologic malignancies. *Blood*, 97(8):2434-9 (2001).

# LeukoStrat®

CDx *FLT3* Mutation Assay (CE-marked)

Gel and Capillary CE IVD Assays and IVD Assays




Depicted is a representation of the *FLT3* juxtamembrane (JM) region (TM = transmembrane) and the activating loop of the tyrosine kinase (TK) domain. Black arrows represent the relative positions of primers that target in and around the JM region for ITD or the activating loop of the kinase domain for TKD. Colored dots represent fluorophores on labeled primers. The yellow box has vertical black lines that represent the position of the EcoRV restriction digest sites.

## Reagents

| Controls | Units in Assay |
|---|---|
| *FLT3* Extraction Control | 1 x 1800 µL tube |
| *FLT3* ITD Master Mix | 1 x 1500 µL tube |
| *FLT3* TKD Master Mix | 1 x 1500 µL tube |
| *FLT3* ITD Positive Control | 1 x 100 µL tube |
| *FLT3* TKD Positive Control | 1 x 100 µL tube |
| *FLT3* No Template Control | 1 x 200 µL tube |

All reagents should be stored at -15 to -30 degrees C.

## Ordering Information

| Catalog # | Products | Quantity |
|---|---|---|
| K-412-0291 | LeukoStrat® CDx *FLT3* Mutation Assay | 33 reactions |
| K-412-0281 | LeukoStrat® CDx *FLT3* Mutation Assay Software | 1 CD complimentary with purchase |

These are *in vitro* diagnostic products, and are not available for sale or use within North America.

# CDx *FLT3* Mutation Assay - AUS

**The only internationally standardized IVD assay for *FLT3* Signal Ratio mutation analysis for selection of acute myeloid leukemia (AML) patients eligible for treatment with midostaurin.**

## Intended Use

The LeukoStrat® CDx *FLT3* Mutation Assay is a PCR-based in vitro diagnostic test designed to detect internal tandem duplications (ITD) and tyrosine kinase domain (TKD) mutations D835 and I836 in the *FLT3* gene in genomic DNA extracted from mononuclear cells obtained from peripheral blood or bone marrow aspirates of patients diagnosed with acute myelogenous leukemia (AML).

In regions where midostaurin is available, the LeukoStrat® CDx *FLT3* Mutation Assay is used as an aid in the selection of patients with AML for whom midostaurin treatment is being considered.

## Summary and Explanation of the Test

AML in general has a poor prognosis. Assessment of the mutation status of the *FLT3* (fms related tyrosine kinase 3) receptor gene in karyotype normal AML is the most important prognostic indicator of disease outcome, which is often substantial, as many studies in AML have shown that the presence of *FLT3* activating mutations portends a poor prognosis.[1,2] The LeukoStrat CDx *FLT3* Mutation Assay targets regions of the *FLT3* gene to identify ITD mutations and TKD mutations, such as the D835 and I836 mutations, and has been validated in an international clinical trial.

The LeukoStrat CDx *FLT3* Mutation Assay includes reagents, equipment, software and procedures for isolating mononuclear cells and extracting DNA from patient specimens to determine if *FLT3* mutations are present. DNA is amplified via PCR and the amplicons are detected via capillary electrophoresis. *FLT3* mutation status is determined by the LeukoStrat CDx *FLT3* Software. A *FLT3* ITD and/or TKD mutation is reported as Positive if the mutant:wild-type signal ratio meets or exceeds the clinical cutoff of 0.05.

## Method Description

**ITD Mutations of *FLT3***

The LeukoStrat CDx *FLT3* Mutation Assay uses fluorescently labeled primers that are in the JM region. Wild-type *FLT3* alleles will amplify and produce a product at 327±1 bp as measured by this assay, while alleles that contain ITD mutations will produce a product that exceeds 330±1 bp (please see Figure, right).

**TKD Mutations of *FLT3***

The LeukoStrat CDx *FLT3* Mutation Assay uses primers that lie on either side of the TKD region. The *FLT3* target region is amplified using PCR and then an EcoRV restriction digest is performed. Wild-type alleles of the *FLT3* gene yield digestion products of 79±1 bp whereas mutant alleles yield products of 125±1 bp or 127±1 bp from the original undigested amplicon product of 145±1 bp or 147±1 bp, as measured by this assay (please see Figure, right).

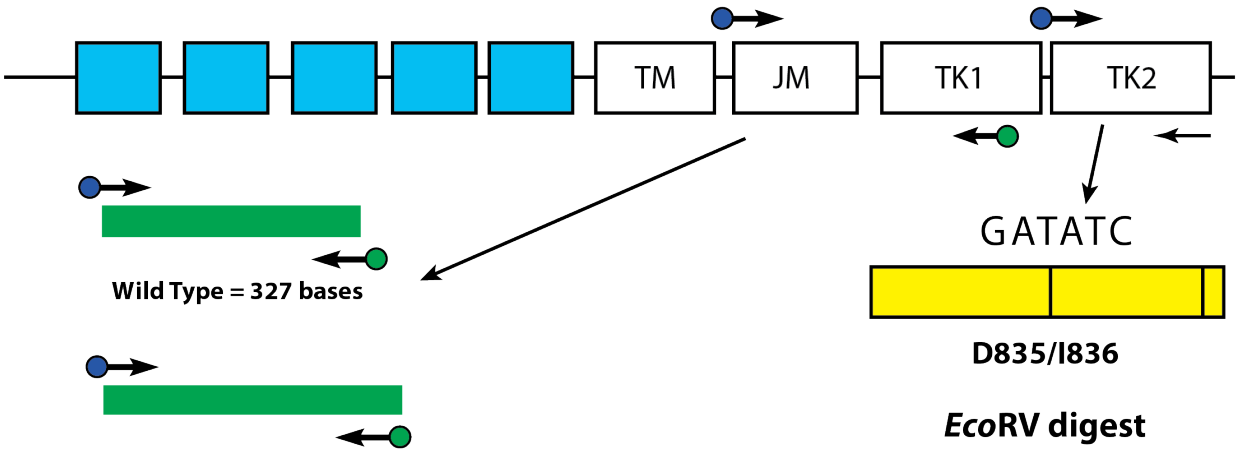
## Reference

1. Murphy KM et al., A Clinical PCR/Capillary Electrophoresis Assay for the Detection of Internal Tandem Duplication and Point Mutation of the *FLT3* Gene. *J. Mol. Diag.* 5:96-102 (2003).
2. Yamamoto, Y., et al., Activating mutation of D835 within the activation loop of *FLT3* in human hematologic malignancies. *Blood*, 97(8):2434-9 (2001).

# LeukoStrat®

CDx *FLT3* Mutation Assay - AUS

Gel and Capillary CE IVD Assays and IVD Assays




Depicted is a representation of the *FLT3* juxtamembrane (JM) region (TM = transmembrane) and the activating loop of the tyrosine kinase (TK) domain. Black arrows represent the relative positions of primers that target in and around the JM region for ITD or the activating loop of the kinase domain for TKD. Colored dots represent fluorophores on labeled primers. The yellow box has vertical black lines that represent the position of the EcoRV restriction digest sites.

## Reagents

| Controls | Units in Assay |
|---|---|
| *FLT3* Extraction Control | 1 x 1800 µL tube |
| *FLT3* ITD Master Mix | 1 x 1500 µL tube |
| *FLT3* TKD Master Mix | 1 x 1500 µL tube |
| *FLT3* ITD Positive Control | 1 x 100 µL tube |
| *FLT3* TKD Positive Control | 1 x 100 µL tube |
| *FLT3* No Template Control | 1 x 200 µL tube |

All reagents should be stored at -15 to -30 degrees C.

## Ordering Information

| Catalog # | Products | Quantity |
|---|---|---|
| K-412-0381 | LeukoStrat® CDx *FLT3* Mutation Assay | 33 reactions |
| K-412-0391 | LeukoStrat® CDx *FLT3* Mutation Assay Software | 1 CD complimentary with purchase |

These are *in vitro* diagnostic products, and are available for sale only within Australia.

# LeukoStrat®

リューコストラット CDX FLT3 変異検査

Gel and Capillary CE IVD Assays and IVD Assays

## Available in Japan

# CDx *FLT3* Mutation Assay

**The only internationally standardized assay for *FLT3* Signal Ratio mutation analysis for assessment of acute myeloid leukemia (AML) patients eligible for treatment with Gilteritinib Fumarate or Quizartinib Hydrochloride.**

## Intended Use

The LeukoStrat CDx *FLT3* Mutation Assay is a PCR-based, *in vitro* diagnostic test designed to detect internal tandem duplication (ITD) mutations and tyrosine kinase domain (TKD) mutations D835 and I836 in the *FLT3* gene in genomic DNA extracted from mononuclear cells obtained from peripheral blood or bone marrow aspirates of patients diagnosed with acute myelogenous leukemia.

The LeukoStrat CDx *FLT3* Mutation Assay is used as an aid in the assessment of patients with AML for whom Gilteritinib Fumarate treatment is being considered.

The LeukoStrat CDx *FLT3* Mutation Assay is used as an aid in the assessment of patients with AML for whom Quizartinib Hydrochloride treatment is being considered.

## Summary and Explanation of the Test

AML in general has a poor prognosis. Assessment of the mutation status of the *FLT3* (fms related tyrosine kinase 3) receptor gene in karyotype normal AML is the most important prognostic indicator of disease outcome, which is often substantial, as many studies in AML have shown that the presence of *FLT3* activating mutations portends a poor prognosis.[1,2] The LeukoStrat CDx *FLT3* Mutation Assay targets regions of the *FLT3* gene to identify ITD mutations and TKD mutations, such as the D835 and I836 mutations, and has been validated in an international clinical trial.

The LeukoStrat CDx *FLT3* Mutation Assay includes reagents, equipment, software and procedures for isolating mononuclear cells and extracting DNA from patient specimens to determine if *FLT3* mutations are present. DNA is amplified via PCR and the amplicons are detected via capillary electrophoresis. *FLT3* mutation status is determined by the LeukoStrat CDx *FLT3* Software. A *FLT3* ITD and/or TKD mutation is reported as Positive if the mutant:wild-type signal ratio meets or exceeds the clinical cutoff of 0.05.

## Method Description

**ITD Mutations of *FLT3***

The LeukoStrat CDx *FLT3* Mutation Assay uses fluorescently labeled primers that are in the JM region. Wild-type *FLT3* alleles will amplify and produce a product at 327±1 bp as measured by this assay, while alleles that contain ITD mutations will produce a product that exceeds 330±1 bp (please see Figure, right).

**TKD Mutations of *FLT3***

The LeukoStrat CDx *FLT3* Mutation Assay uses primers that lie on either side of the TKD region. The *FLT3* target region is amplified using PCR and then an EcoRV restriction digest is performed. Wild-type alleles of the *FLT3* gene yield digestion products of 79±1 bp whereas mutant alleles yield products of 125±1 bp or 127±1 bp from the original undigested amplicon product of 145±1 bp or 147±1 bp, as measured by this assay (please see Figure, right).

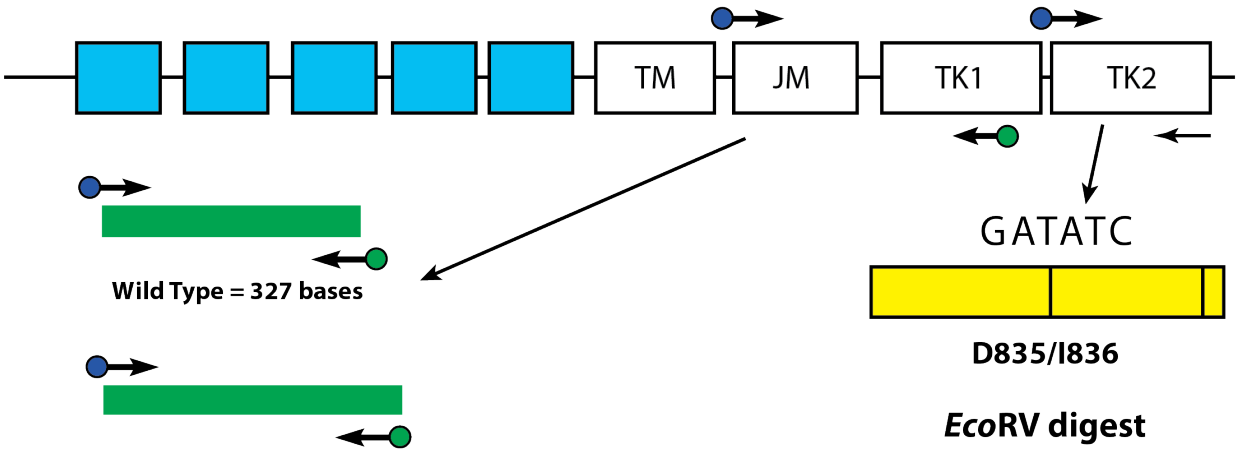
## Reference


1. Murphy KM et al., A Clinical PCR/Capillary Electrophoresis Assay for the Detection of Internal Tandem Duplication and Point Mutation of the *FLT3* Gene. *J. Mol. Diag.* 5:96-102 (2003).
2. Yamamoto, Y., et al., Activating mutation of D835 within the activation loop of *FLT3* in human hematologic malignancies. *Blood,* 97(8):2434-9 (2001).

# LeukoStrat®

リューコストラット CDX FLT3 変異検査

Gel and Capillary CE IVD Assays and IVD Assays




Depicted is a representation of the *FLT3* juxtamembrane (JM) region (TM = transmembrane) and the activating loop of the tyrosine kinase (TK) domain. Black arrows represent the relative positions of primers that target in and around the JM region for ITD or the activating loop of the kinase domain for TKD. Colored dots represent fluorophores on labeled primers. The yellow box has vertical black lines that represent the position of the EcoRV restriction digest sites.

## Reagents

| Reagent Name | Units in Assay |
|---|---|
| *FLT3* Extraction Control | 1 x 1800 µL tube |
| *FLT3* ITD Master Mix | 1 x 1500 µL tube |
| *FLT3* TKD Master Mix | 1 x 1500 µL tube |
| *FLT3* ITD Positive Control | 1 x 100 µL tube |
| *FLT3* TKD Positive Control | 1 x 100 µL tube |
| *FLT3* No Template Control | 1 x 200 µL tube |
| Taq DNA Polymerase Enzyme | 1 x 200 µL tube |
| EcoRV Enzyme | 1 x 200 µL tube |

All reagents should be stored at -15 to -30 degrees C.

## Ordering Information

| Catalog # | Products | Quantity |
|---|---|---|
| K-412-0331 | LeukoStrat® CDx *FLT3* Mutation Assay (Japan) | 33 reactions |
| K-412-0341 | LeukoStrat® CDx *FLT3* Mutation Assay Software (Japan) | 1 CD complimentary with purchase |

These are *in vitro* diagnostic products, and are available for sale or use within Japan.

# Gel and Capillary

## Research Use Only (RUO) Assay

### B-Cell Assays

94/ *IGH + IGK* B-Cell Clonality Assays

96 / *IGH* Gene Rearrangement Assays

98/ *IGH* Gene Clonality Assays

100/ *IGK* Gene Clonality Assays

102 / *IGL* Gene Clonality Assays

### T-Cell Assays

104 / *TCRB + TCRG* T-Cell Clonality Assays

106 / *TCRB* Gene Clonality Assays

108 / T-Cell Receptor Gama Gene Rearrangement Assays 2.0

110 / T-Cell Receptor Gama Gene Rearrangement Assays

112/ *TCRG* Gene Clonality Assays

114 / *TCRD* Gene Clonality Assays

### Translocation Assays

116 / *BCL1*/JH Translocation Assay

118 / *BCL2*/JH t(14;18) Translocation Assay

120/ *BCL2*/JH Translocation Assay

122/ *BCL2*/JH t(9;22) Translocation Assays

124/ PML/RAR*α* t(15;17) Translocation Assays

### Mutation Assays

126/ *IGH* Somatic Hypermutation Assays v2.0

128/ *FLT3* Mutation Assays

# Gel and Capillary

## Invivoscribe offers an array of assays for B- and T-cell gene clonality/rearrangements, mutations, and chromosome translocations for the study of hematologic malignancies.

These (RUO) assays are available for either ABI capillary electrophoresis fluorescence, or PAGE/agarose gel detection, and contain the PCR master mixes, recommended controls, and Instructions For Use.

On the following pages, you will find detailed information on each RUO assay, including: assay use, background information, typical output data, kit contents, and ordering information. These assays are available in regular sizes (30 or 33 reactions) or high-volume MegaKit formats (300 or 330 reactions).

These pages contain Research Use Only products which are not for use in diagnostic procedures. Research Use Only (RUO) assays are not for sale in Europe and other global markets where equivalent CE-IVD assays are available and registered with the appropriate regulatory agencies. Refer to the preceding pages for information regarding our IdentiClone® and LeukoStrat® CE-IVD Assays.

For more information, please visit www.invivoscribe.com

# Gel and Capillary

*IGH + IGK* B-Cell Clonality Assays

Research Use Only (RUO) Assay

# *IGH + IGK* B-Cell Clonality Assays

## Assay Use

*IGH + IGK* B-Cell Clonality Assays are useful for studies involving:

- Identification of clonal B-cell populations highly suggestive of B-cell malignancies
- Lineage determination of leukemias and lymphomas
- Monitoring and evaluation of disease recurrence
- Detection and assessment of residual disease
- Evaluation of new research and methods in malignancy studies

## Summary and Explanation of the Test

Five PCR master mixes are included in these test kits to test for rearrangements of both *IGH* and *IGK*. *IGH* Tubes A, B, and C target the conserved framework 1, 2, and 3 regions (respectively) within the variable ($V_H$) region and the joining ($J_H$) region of the *IGH* locus. *IGK* tubes A and B target the variable ($V_\kappa$), intragenic and joining ($J_\kappa$), and kappa deleting element ($K_{de}$) regions of the *IGK* locus.

Positive and negative controls, as well as Specimen Control Size Ladder Master Mix are included. PCR products can be analyzed by capillary electrophoresis or heteroduplex analysis. Clonality is indicated if any one of the master mixes generates clonal products.

## Background

The immunoglobulin heavy chain (*IGH*) gene locus on chromosome 14 (14q32.33, formerly 14q32.3) includes 46–52 functional and 30 nonfunctional variable ($V_H$), 27 functional diversity ($D_H$), and 6 functional joining ($J_H$) gene segments spread over 1250 kilobases.[1,2] The most frequently used $V_H$ gene segments in normal and malignant B cells belong to $V_H3$, $V_H4$, and $V_H1$ families, which together cover 75–95% of $V_H$ usage. The $V_H$ gene segments contain three framework regions (FR) and two complementarity determining regions (CDR). The FRs are characterized by their similarity among the various $V_H$ segments, whereas the CDRs are highly different even within the same $V_H$ family. The CDRs represent the preferred target sequences for somatic hypermutations; however, somatic mutations can also occur in the FRs. Therefore, family-specific primers in the three different FRs were designed to increase the detection rate of clonal *IGH* B-cell populations and decrease the occurrence of false-negative results due to somatic hypermutation in primer binding sites.[1]

The human immunoglobulin kappa (*IGK*) light chain locus on the short arm of chromosome 2 (2p11.2) spans 1820 kb. It is made up of 76 variable ($V_\kappa$) gene segments belonging to seven subgroups, five joining ($J_\kappa$) gene segments, and one constant ($C_\kappa$) gene segment. Productive assembly of the kappa gene is successful in about 60% of human B lymphocytes[2]; however, even when unsuccessful, clonal B cells generally retain the rearranged kappa genes. The $V_\kappa$ segments encode the first 95 N-terminal amino acids. Positions 96–108 are encoded by one of five joining ($J_\kappa$) gene segments. The constant ($C_\kappa$) portion of the kappa light chain (amino acids 109–214) is encoded by a single constant ($C_\kappa$) region separated from the $J_\kappa$ region by an intron.

The length of the hypervariable CDR3 in kappa light chain genes is limited and rearrangements in this region display significant skewing (platykurtosis).[3] Therefore, clonal CDR3 products generated from this region are easily and reliably identified by heteroduplex analysis or capillary electrophoresis.

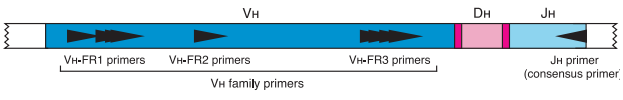
## Specimen Requirements

- 5 mL of peripheral blood, bone marrow biopsy, or bone marrow aspirate anti-coagulated with heparin or EDTA; or,
- Minimum 5 mm cube of tissue; or,
- 3 µg of genomic DNA; or,
- Formalin-fixed, paraffin-embedded (FFPE) tissue or slides.

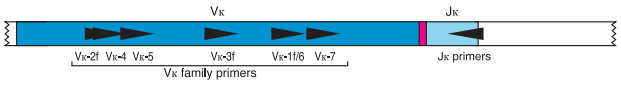
## Reference


1. M Hummel et al., *Leukemia* 17: 2266–2272 (2003).
2. AW Langerak et al., *Leukemia* 17: 2272–2275 (2003).
3. EP Rock, PR Sibbald, MM Davis, and YH Chien. *J. Exp. Med.* 179(1): 323–328 (1994).
4. JJM van Dongen et al., *Leukemia* 17: 2257–2317 (2003).

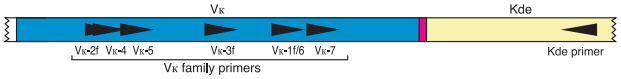

EuroClonality **This assay is based on the EuroClonality/BIOMED-2 Concerted Action BMH4-CT98-3936.**




*IGH* Tube A: 6 VH-FR1 Primers + JH Consensus Primer
*IGH* Tube B: 7 VH-FR2 Primers + JH Consensus Primer
*IGH* Tube C: 7 VH-FR3 Primers + JH Consensus Primer




*IGK* tube A: 6 Vκ primers + 2 Jκ primers




*IGK* tube B: 6 Vκ primers and INTR primer + 1 κde primer


Figure Legend: Simple representation of the organization of a rearranged immunoglobulin heavy chain (*IGH*) gene on chromosome 14 and the immunoglobulin kappa light chain gene on chromosome 2p11.2. Black arrows represent the relative positions of primers that target the conserved framework regions (FR1–3) and the downstream consensus $J_H$ gene segments for *IGH* and the $V_\kappa$, $J_\kappa$, INTR and $K_{de}$ primers which are included in the *IGK* master mix tubes.

# Gel and Capillary

*IGH + IGK* B-Cell Clonality Assays

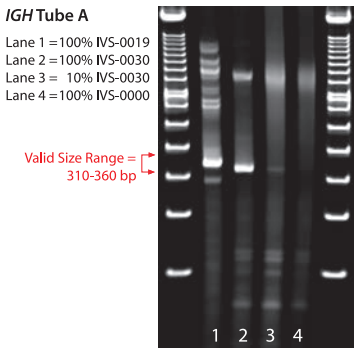
Research Use Only (RUO) Assay

## Reagents

| Controls | Concentration | Units in Assay | Units in Assay MegaKit |
|---|---|---|---|
| IVS-0030 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0019 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0007 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0000 Polyclonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| **Master Mixes** | **Target** | **Units in Assay** | **Units in Assay MegaKit** |
| *IGH* Tube A | Framework 1 + $J_H$ | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *IGH* Tube B | Framework 2 + $J_H$ | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *IGH* Tube C | Framework 3 + $J_H$ | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *IGK* Tube A | Vκ-Jκ | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *IGK* Tube B | Vκ-$K_{de}$ | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| Specimen Control Size Ladder | Multiple Genes | 1 x 1500 µL tube | 10 x 1500 µL tubes |

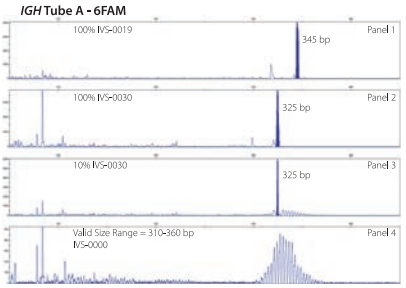
## Gel Electrophoresis Detection

The data shown was generated using the master mix indicated. Amplified products were heteroduplexed and then run on a 6% non-denaturing polyacrylamide/TBE gel. Lane 1 is data generated testing an alternative 100% clonal control DNA; lane 2 is generated testing the recommended 100% clonal control DNA; lane 3 is data generated testing a 10% dilution of the recommended clonal control DNA; and, lane 4 is data generated testing IVS-0000 Polyclonal Control DNA (Cat# 4-092-0010). This DNA is used as a negative control for many of our tests. A standard 100 base pair DNA size ladder was run in the lanes flanking the test samples.




## Capillary Electrophoresis Detection (ABI)

The data shown was generated using the master mix indicated. Amplified products were run on an ABI 3100 instrument. For the master mix: Panel 1 displays data generated testing an alternative 100% clonal control DNA; panel 2 displays data generated testing the recommended 100% clonal control DNA; panel 3 displays data generated testing a 10% dilution of the recommended clonal control DNA; and, panel 4 displays data generated testing the IVS-0000 Polyclonal Control DNA (Cat# 4-092-0010). Our IVS-0000 DNA is often tested to provide information regarding valid size ranges for each master mix.




## Ordering Information

| Catalog # | Products | Quantity |
|---|---|---|
| 1-100-0010 | *IGH* + *IGK* B-Cell Clonality Assay - Gel Detection | 33 reactions |
| 1-100-0020 | *IGH* + *IGK* B-Cell Clonality Assay MegaKit - Gel Detection | 330 reactions |
| 1-100-0031 | *IGH* + *IGK* B-Cell Clonality Assay - ABI Fluorescence Detection | 33 reactions |
| 1-100-0041 | *IGH* + *IGK* B-Cell Clonality Assay MegaKit - ABI Fluorescence Detection | 330 reactions |

# *IGH* Gene Rearrangement Assays

## Assay Use

*IGH* Gene Rearrangement Assays are useful for studies involving:

- Identification of clonal B-cell populations highly suggestive of B-cell malignancies
- Lineage determination of leukemias and lymphomas
- Monitoring and evaluation of disease recurrence
- Detection and assessment of residual disease
- Evaluation of new research and methods in malignancy studies

## Summary and Explanation of the Test

Genomic DNA is amplified using three PCR master mixes that target the three conserved framework regions (FR1, FR2, and FR3) of the *IGH* gene and the joining (JH) region. These regions flank the unique, hypervariable, antigen-binding, complementarity determining region 3 (CDR3). All positive and negative DNA controls, as well as an Amplification Control master mix, are included. The limit of detection of this assay is one clonal B cell in a background of a hundred normal cells. PCR products can be analyzed by capillary electrophoresis or standard gel electrophoresis with ethidium bromide staining. Clonality is indicated if one or more of the three framework master mixes generates clonal products.

## Background

Genes encoding immunoglobulin heavy chain (*IGH*) molecules are assembled from multiple polymorphic gene segments that undergo rearrangement and selection during B-cell development.[2] Rearrangement of these variable (VH), diversity (DH), and joining (JH) genetic segments result in VDJ products of unique length and sequence.[1,2] Clonal *IGH* rearrangements can be rapidly identified through analyses of the size distributions of DNA products amplified from conserved sequences that flank this region.[2] For example, DNA isolated from a normal polyclonal population of B cells produces a Gaussian distribution (bell-shaped size curve) of amplified products; whereas, DNA amplified from a clonal B-cell population generates one or two product(s) of unique size that reflect proliferation of a single rearranged clone.[1] In comparison, southern blot analysis requires 1-2 weeks, is significantly less sensitive, and requires approximately one hundred times more DNA than PCR-based assays, which can be completed in 4-5 hours.[1] In addition, tests of samples previously designated Quantity Not Sufficient (QNS), such as formalin-fixed, paraffin embedded (FFPE) tissue sections, routinely produce a valid result with PCR methods.

## Specimen Requirements

- 5 mL of peripheral blood, bone marrow biopsy, or bone marrow aspirate anti-coagulated with heparin or EDTA; or,
- Minimum 5 mm cube of tissue; or,
- 2 µg of genomic DNA; or,
- Formalin-fixed, paraffin-embedded tissue or slides.

## Reference

1. JE Miller, SS Wilson, DL Jaye, and M Kronenberg. *J. Mol. Diag.* 4: 101-117 (1999).
2. S Tonegawa. *Nature* 302: 575-581 (1983).

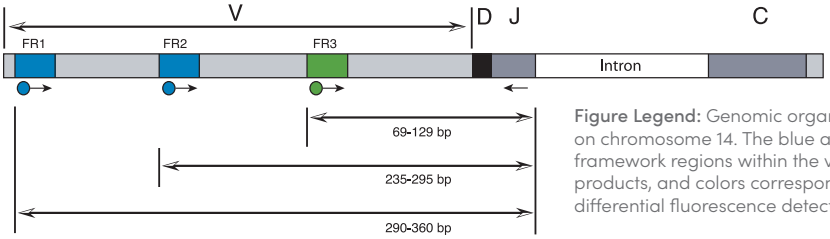



**Figure Legend:** Genomic organization of a rearranged immunoglobulin heavy chain gene on chromosome 14. The blue and green arrows represent primers targeting the conserved framework regions within the variable region gene. The relative location, size range of valid products, and colors correspond to the products generated from each of these regions when differential fluorescence detection methods are used.

# Gel and Capillary

*IGH* Gene Rearrangement Assays

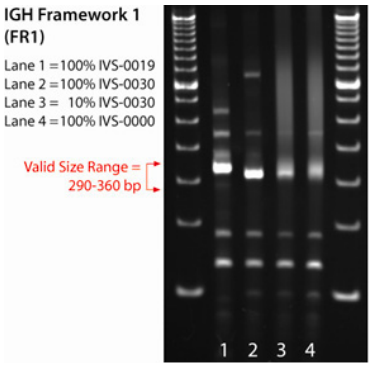
Research Use Only (RUO) Assay

## Reagents

| Controls | Concentration | Units in Assay | Units in Assay MegaKit |
|---|---|---|---|
| IVS-0030 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0029 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0000 Polyclonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| **Master Mixes** | **Target** | **Units in Assay** | **Units in Assay MegaKit** |
| *IGH* Framework 1 | Framework 1 + JH | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *IGH* Framework 2 | Framework 2 + JH | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *IGH* Framework 3 | Framework 3 + JH | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| Amplification Control | *HLA-DQa* | 1 x 1500 µL tube | 10 x 1500 µL tubes |

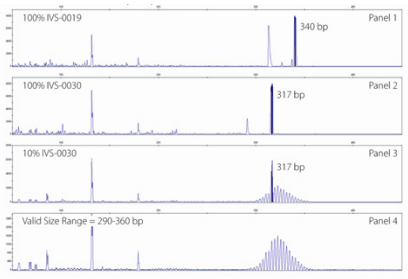
## Gel Electrophoresis Detection

The data shown was generated using the master mix indicated. Amplified products were run on a 6% non-denaturing polyacrylamide/TBE gel. Lane 1 is data generated testing an alternative 100% clonal control DNA; lane 2 is data generated testing the recommended 100% clonal control DNA; lane 3 is data generated testing a 10% dilution of the recommended clonal control DNA; and, lane 4 is data generated testing IVS-0000 Polyclonal Control DNA (Cat# 4-092-0010). IVS-0000 is used as a negative control for many of our tests. A standard 100 base pair DNA size ladder was run in the lanes flanking the test samples.




## Capillary Electrophoresis Detection (ABI)

The data shown was generated using the master mix indicated. Amplified products were run on an ABI 3100 instrument. Panel 1 displays data generated testing an alternative 100% clonal control DNA; panel 2 displays data generated testing the recommended 100% clonal control DNA; panel 3 displays data generated testing a 10% dilution of the recommended clonal control DNA; and, panel 4 displays data generated testing the IVS-0000 Polyclonal Control DNA (Cat# 4-092-0010). Our IVS-0000 DNA is often tested to provide information regarding valid size ranges for each master mix.




## Ordering Information

| Catalog # | Products | Quantity |
|---|---|---|
| 1-101-0010 | *IGH* Gene Rearrangement Assay - Gel Detection | 30 reactions |
| 1-101-0030 | *IGH* Gene Rearrangement Assay MegaKit - Gel Detection | 300 reactions |
| 1-101-0051 | *IGH* Gene Rearrangement Assay - ABI Fluorescence Detection | 30 reactions |
| 1-101-0071 | *IGH* Gene Rearrangement Assay MegaKit - ABI Fluorescence Detection | 300 reactions |

# *IGH* Gene Clonality Assays

## Assay Use

*IGH* Gene Clonality Assays are useful for studies involving:

- Identification of clonal B-cell populations highly suggestive of B-cell malignancies
- Lineage determination of leukemias and lymphomas
- Monitoring and evaluation of disease recurrence
- Detection and assessment of residual disease
- Evaluation of new research and methods in malignancy studies

## Summary and Explanation of the Test

Five master mixes target conserved regions within the variable (VH), diversity (DH), and the joining (JH) regions that flank the unique hypervariable, antigen-binding, complementarity determining region 3 (CDR3). Tube A contains six framework region 1 (FR1) primers and a consensus JH region primer. Tube B contains seven framework region 2 (FR2) primers and a consensus JH primer. Tube C contains seven framework region 3 (FR1) primers and a consensus JH primer. Tube D contains six DH region primers and a consensus JH region primer. Tube E contains a DH7 region primer and a consensus JH primer. Positive and negative controls, as well as the Specimen Control Size Ladder Master Mix are included. PCR products can be analyzed by capillary electrophoresis or heteroduplex analysis. Clonality is indicated if any one of the master mixes generates a clonal product.

## Background

The immunoglobulin heavy chain (*IGH*) gene locus on chromosome 14 (14q32.33, formerly 14q32.3) includes 46–52 functional and 30 nonfunctional variable (VH), 27 functional diversity (DH), and 6 functional joining (JH) gene segments spread over 1250 kilobases.[1,2] The most frequently used VH gene segments in normal and malignant B cells belong to the VH3, VH4, and VH1 family, together covering 75–95% of VH usage. The VH gene segments contain three framework regions (FR) and two complementarity determining regions (CDR).

The FRs are characterized by their similarity among the various VH segments, whereas the CDRs are highly different even within the same VH family. The CDRs represent the preferred target sequences for somatic hypermutations; however, somatic mutations can also occur in the FRs. Therefore, family-specific primers in the three different FRs were designed to increase the detection rate of clonal *IGH* B-cell populations and decrease the occurrence of false-negative results due to somatic hypermutation in primer binding sites.[1] In addition to VH-JH rearrangements, incomplete DH-JH rearrangements have been found in mature and immature B-cell malignancies. Therefore, DH-JH PCR analysis may be of added value for clonality assessment.[2]

## Specimen Requirements

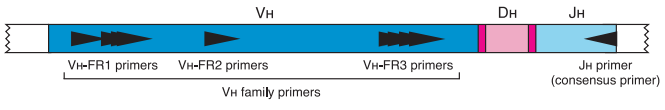
- 5 mL of peripheral blood, bone marrow biopsy, or bone marrow aspirate anti-coagulated with heparin or EDTA; or,
- Minimum 5 mm cube of tissue; or,
- 3 µg of genomic DNA; or,
- Formalin-fixed, paraffin-embedded (FFPE) tissue or slides.

## Reference

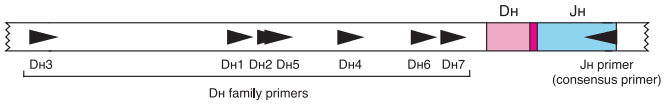
1. M Hummel et al., *Leukemia* 17:2266-2272 (2003).
2. AW Langerak et al., *Leukemia* 17:2272-2275 (2003).
3. JJM van Dongen et al., *Leukemia* 17:2257-2317 (2003).

EuroClonality This assay is based on the **EuroClonality/BIOMED-2** Concerted Action BMH4-CT98-3936.

**Figure Legend:** Simple representation of the organization of a rearranged immunoglobulin heavy chain gene on chromosome 14. Black arrows represent the relative positions of primers that target the conserved framework (FR1-3) and diversity (DH1-7) regions, and the downstream consensus JH gene segments. The amplicon products generated from each of these regions can be differentially detected when fluorescent primer sets are used with capillary electrophoresis instruments that employ differential fluorescence detection.




Tube A: 6 $V_H$ -FR1 Primers + $J_H$ Consensus Primer
Tube B: 7 $V_H$ -FR2 Primers + $J_H$ Consensus Primer
Tube C: 7 $V_H$ -FR3 Primers + $J_H$ Consensus Primer




Tube D: 6 $D_H$ Primers + $J_H$ Consensus Primer
Tube E: $D_H$ 7 Primer + $J_H$ Consensus Primer

# Gel and Capillary

*IGH* Gene Clonality Assays

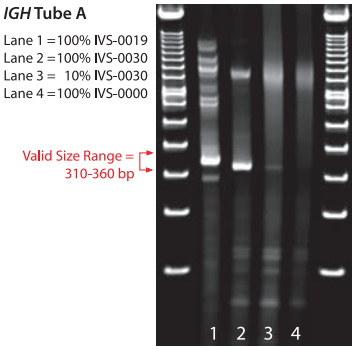
Research Use Only (RUO) Assay

## Reagents

| Controls | Concentration | Units in Assay | Units in Assay MegaKit |
|---|---|---|---|
| IVS-0030 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0019 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0024 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0008 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0000 Polyclonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| **Master Mixes** | **Target** | **Units in Assay** | **Units in Assay MegaKit** |
| *IGH* Tube A | Framework 1 + JH | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *IGH* Tube B | Framework 2 + JH | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *IGH* Tube C | Framework 3 + JH | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *IGH* Tube D | DH1-6 + JH | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *IGH* Tube E | DH7 + JH | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| Specimen Control Size Ladder | Multiple Genes | 1 x 1500 µL tube | 10 x 1500 µL tubes |

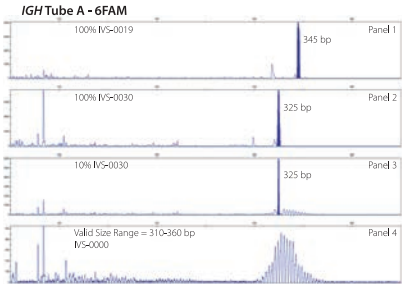
## Gel Electrophoresis Detection

The data shown was generated using the master mix indicated. Amplified products were heteroduplexed and then run on a 6% non-denaturing polyacrylamide/TBE gel. Lane 1 is data generated testing an alternative 100% clonal control DNA; lane 2 data is generated testing the recommended 100% clonal control DNA; lane 3 is data generated testing a 10% dilution of the recommended clonal control DNA; and, lane 4 is data generated testing IVS-0000 Polyclonal Control DNA (Cat# 4-092-0010). IVS-0000 is used as a negative control for many of our tests. A standard 100 base pair DNA size ladder was run in the lanes flanking the test samples.




## Capillary Electrophoresis Detection (ABI)

The data shown was generated using the master mix indicated. Amplified products were run on an ABI 3100 instrument. For the master mix: Panel 1 displays data generated testing an alternative 100% clonal control DNA; panel 2 displays data generated testing the recommended 100% clonal control DNA; panel 3 displays data generated testing a 10% dilution of the recommended clonal control DNA; and, panel 4 displays data generated testing the IVS-0000 Polyclonal Control DNA (Cat# 4-092-0010). Our IVS-0000 DNA is often tested to provide information regarding valid size ranges for each master mix.




## Ordering Information

| Catalog # | Products | Quantity |
|---|---|---|
| 1-101-0020 | *IGH* Gene Clonality Assay - Gel Detection | 33 reactions |
| 1-101-0040 | *IGH* Gene Clonality Assay MegaKit - Gel Detection | 330 reactions |
| 1-101-0061 | *IGH* Gene Clonality Assay - ABI Fluorescence Detection | 33 reactions |
| 1-101-0081 | *IGH* Gene Clonality Assay MegaKit - ABI Fluorescence Detection | 330 reactions |

# *IGK* Gene Clonality Assays

## Assay Use

*IGK* Gene Clonality Assays are useful for studies involving:

- Identification of clonal B-cell populations highly suggestive of B-cell malignancies
- Lineage determination of leukemias and lymphomas
- Monitoring and evaluation of disease recurrence
- Detection and assessment of residual disease
- Evaluation of new research and methods in malignancy studies

## Summary and Explanation of the Test

Two master mixes target conserved regions within the variable (Vκ1-7) and the joining (Jκ1-5) regions that flank the unique hypervariable, antigen-binding, complementarity determining region 3 (CDR3). Other primers target the $K_{de}$ and intragenic regions.

Tube A contains six upstream primers and two Jκ region primers. Tube B contains six upstream Vκ region primers, an upstream intragenic primer and a downstream $K_{de}$ primer. Positive and negative controls, as well as a Specimen Control Size Ladder Master Mix, are included. PCR products can be analyzed by capillary electrophoresis or heteroduplex analysis. Clonality is indicated if any one of the master mixes generates clonal products.

## Background

The human immunoglobulin kappa (*IGK*) light chain locus on the short arm of chromosome 2 (2p12, formerly 2p11.2) spans 1820 kb. It is made up of 76 variable (Vκ) gene segments belonging to 7 subgroups, 5 joining (Jκ) gene segments, and one constant (Cκ) gene segment. Productive assembly of the kappa gene is successful in about 60% of human B lymphocytes.[1] However, even when unsuccessful, clonal B cells generally retain the rearranged kappa genes. The Vκ segments encode the first 95 N-terminal amino acids. Positions 96-108 are encoded by one of five joining (Jκ) gene segments. The constant (Cκ) portion of the kappa light chain (amino acids 109-214) is encoded by a single constant (Cκ) region separated from the Jκ region by an intron. The length of the hypervariable complementarity determining region 3 (CDR3) in kappa light chain genes is limited and rearrangements in this region display significant skewing (platykurtosis).[2]

Therefore, clonal CDR3 products generated from this region are most easily and reliably identified by heteroduplex analysis using standard polyacrylamide gels. Alternatively, capillary electrophoresis or gene sequencing instruments coupled with differential fluorescence detection can be used for analysis.

## Specimen Requirements

- 5 mL of peripheral blood, bone marrow biopsy, or bone marrow aspirate anti-coagulated with heparin or EDTA; or,
- Minimum 5 mm cube of tissue; or,
- 2 µg of genomic DNA; or,
- Formalin-fixed, paraffin-embedded (FFPE) tissue or slides.

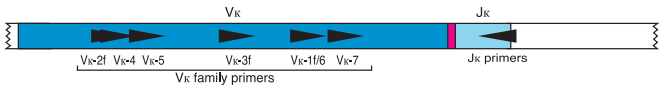
## Reference

1. AW Langerak et al., *Leukemia* 17: 2275-2280 (2003).
2. EP Rock, PR Sibbald, MM Davis, and YH Chien. *J. Exp. Med.* 179(1): 323-328 (1994).
3. JJM van Dongen et al., *Leukemia* 17: 2257-2317 (2003).

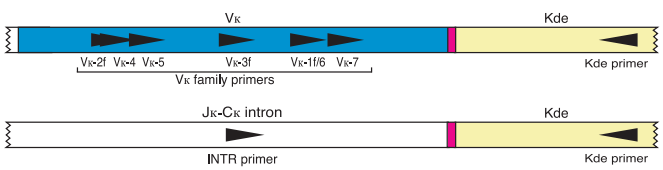
EuroClonality

This assay is based on the **EuroClonality/BIOMED-2** Concerted Action BMH4-CT98-3936.

**Figure Legend:** Schematic diagram of the immunoglobulin kappa light chain gene complex on chromosome 2p11.2. Shown are the relative positions and orientations for the Vκ-Jκ, and $K_{de}$ primers, which are included in the *IGK* master mix tubes.




*IGK* tube A: 6 Vκ primers + 2 Jκ primers




*IGK* tube B: 6 Vκ primers and INTR primer + 1 Kde primer

# Gel and Capillary

*IGK* Gene Clonality Assays

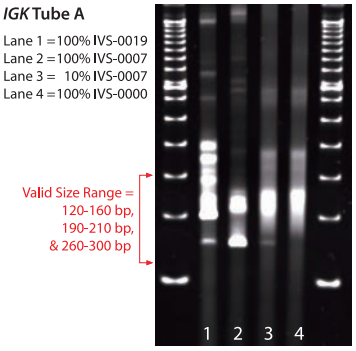
Research Use Only (RUO) Assay

## Reagents

| Controls | Concentration | Units in Assay | Units in Assay MegaKit |
|---|---|---|---|
| IVS-0007 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0000 Polyclonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| **Master Mixes** | **Target** | **Units in Assay** | **Units in Assay MegaKit** |
| *IGK* Tube A | Vκ-Jκ | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *IGK* Tube B | Vκ-$K_{de}$ | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| Specimen Control Size Ladder | Multiple Genes | 1 x 1500 µL tube | 10 x 1500 µL tubes |

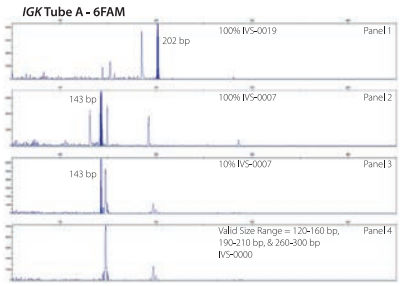
## Gel Electrophoresis Detection

The data shown was generated using the master mix indicated. Amplified products were heteroduplexed and then run on a 6% non-denaturing polyacrylamide/TBE gel. Lane 1 is data generated testing an alternative 100% clonal control DNA; lane 2 is data generated testing the recommended 100% clonal control DNA; lane 3 is data generated testing a 10% dilution of the recommended clonal control DNA; and, lane 4 is data generated testing IVS-0000 Polyclonal Control DNA (Cat# 4-092-0010). The IVS-0000 control is used as a negative control for many of our tests. A standard 100 base pair DNA size ladder was run in the lanes flanking the test samples.




## Capillary Electrophoresis Detection (ABI)

The data shown was generated using the master mix indicated. Amplified products were run on an ABI 3100 instrument. Panel 1 displays data generated testing an alternative 100% clonal control DNA; panel 2 displays data generated testing the recommended 100% clonal control DNA; panel 3 displays data generated testing a 10% dilution of the recommended clonal control DNA; and, panel 4 displays data generated testing the IVS-0000 Polyclonal Control DNA (Cat# 4-092-0010). Our IVS-0000 DNA is often tested to provide information regarding valid size ranges for each master mix.




## Ordering Information

| Catalog # | Products | Quantity |
|---|---|---|
| 1-102-0020 | *IGK* Gene Clonality Assay - Gel Detection | 33 reactions |
| 1-102-0030 | *IGK* Gene Clonality Assay MegaKit - Gel Detection | 330 reactions |
| 1-102-0021 | *IGK* Gene Clonality Assay - ABI Fluorescence Detection | 33 reactions |
| 1-102-0031 | *IGK* Gene Clonality Assay MegaKit - ABI Fluorescence Detection | 330 reactions |

# *IGL* Gene Clonality Assays

## Assay Use

*IGL* Gene Clonality Assays are useful for studies involving:

- Identification of clonal B-cell populations highly suggestive of B-cell malignancies
- Lineage determination of leukemias and lymphomas
- Monitoring and evaluation of disease recurrence
- Detection and assessment of residual disease
- Evaluation of new research and methods in malignancy studies

## Summary and Explanation of the Test

The *IGL* Tube master mix targets conserved regions within the variable (Vλ1-3) and the joining (Jλ1-3) regions that flank the unique, hypervariable, antigen-binding, complementarity determining region 3 (CDR3). Positive and negative controls, as well as a Specimen Control Size Ladder Master Mix, are included. PCR products can be analyzed by capillary electrophoresis or heteroduplex analysis. Clonality is indicated if the master mix generates clonal products.

## Background

The human immunoglobulin lambda (*IGL*) light chain locus is located on the long arm of chromosome 22 (22q11.2) and spans 1050 kilobases. It is made up of 73-74 variable (Vλ) gene segments (spread over 900 kilobases), 7-11 joining (Jλ) gene segments and 7-11 constant (Cλ) gene segments depending on the haplotypes. Of the 73-74 Vλ region genes, only 30-33 are functional and can be grouped into 11 families and 3 clans.[1] The Jλ and Cλ region genes are organized in tandem with a Jλ segment preceding a Cλ gene. Typically there are 7 Jλ-Cλ segments of which four are functional and encode the four Ig lambda isotypes.

*IGL* gene rearrangements are present in 5-10% of Ig kappa B-cell malignancies and in all Ig lambda B-cell malignancies. Therefore, Vλ-Jλ rearrangements potentially represent an attractive extra PCR target for clonality studies to compensate for false-negative *IGH* VH-JH PCR results mainly caused by somatic hypermutations. It should be noted that because of the limited size of the junctional region, it is extremely difficult to distinguish polyclonal from monoclonal rearrangements by running a simple agarose or polyacrylamide gel[1]. Therefore, clonal Vλ-Jλ PCR products are most easily and reliably identified by heteroduplex analysis using standard polyacrylamide gels. Alternatively, capillary electrophoresis or gene sequencing instruments coupled with differential fluorescence detection can be used for analysis.[1]

## Specimen Requirements

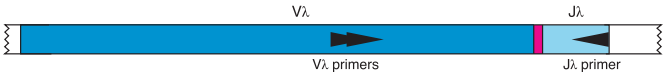
- 5 mL of peripheral blood, bone marrow biopsy, or bone marrow aspirate anti-coagulated with heparin or EDTA; or,
- Minimum 5 mm cube of tissue; or,
- 2 µg of genomic DNA; or,
- Formalin-fixed, paraffin-embedded (FFPE) tissue or slides.

## Reference

1. F Davi et al., *Leukemia* 17:2280-2283 (2003).
2. JJM van Dongen et al., *Leukemia* 17:2257-2317 (2003).

EuroClonality

This assay is based on the **EuroClonality/BIOMED-2** Concerted Action BMH4-CT98-3936.




*IGL* tube: 2 Vλ primers + 1 Jλ primer

**Figure Legend:** Schematic diagram of the immunoglobulin lambda light chain gene complex on chromosome 22q11.2. Shown are the relative positions and orientations for the Vλ and Jλ primers, which are included in the *IGL* master mix tube. The two Vλ primers only target Vλ1, 2, and 3 because these three V families cover approximately 70% of rearrangeable Vλ gene segments, and approximately 90% of all *IGL* gene rearrangements involve these three families. Similarly, the single Jλ primer only targets Jλ1, 2, and 3 because these three J segments are involved in 98% of all *IGL* gene rearrangements.

# Gel and Capillary

*IGL* Gene Clonality Assays

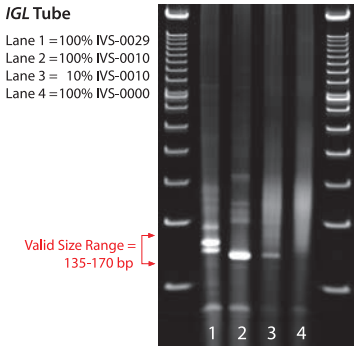
Research Use Only (RUO) Assay

## Reagents

| Controls | Concentration | Units in Assay | Units in Assay MegaKit |
|---|---|---|---|
| IVS-0010 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0029 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0000 Polyclonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| **Master Mixes** | **Target** | **Units in Assay** | **Units in Assay MegaKit** |
| *IGL* Tube | Vλ-Jλ | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| Specimen Control Size Ladder | Multiple Genes | 1 x 1500 µL tube | 10 x 1500 µL tubes |

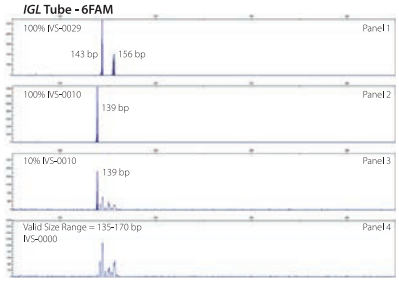
## Gel Electrophoresis Detection

The data shown was generated using the master mix indicated. Amplified products were heteroduplexed and then run on a 6% non-denaturing polyacrylamide/TBE gel. Lane 1 is data generated testing an alternative 100% clonal control DNA; lane 2 is data generated testing the recommended 100% clonal control DNA; lane 3 is data generated testing a 10% dilution of the recommended clonal control DNA; and, lane 4 is data generated testing IVS-0000 Polyclonal Control DNA (Cat# 4-092-0010). The IVS-0000 control is used as a negative control for many of our tests. A standard 100 base pair DNA size ladder was run in the lanes flanking the test samples.




## Capillary Electrophoresis Detection (ABI)

The data shown was generated using the master mix indicated. Amplified products were run on an ABI 3100 instrument. Panel 1 displays data generated testing an alternative 100% clonal control DNA; panel 2 displays data generated testing the recommended 100% clonal control DNA; panel 3 displays data generated testing a 10% dilution of the recommended clonal control DNA; and, panel 4 displays data generated testing the IVS-000 Polyclonal Control DNA (Cat# 4-092-0010). Our IVS-0000 DNA is often tested to provide information regarding valid size ranges for each master mix.




## Ordering Information

| Catalog # | Products | Quantity |
|---|---|---|
| 1-103-0010 | *IGL* Gene Clonality Assay - Gel Detection | 33 reactions |
| 1-103-0020 | *IGL* Gene Clonality Assay MegaKit - Gel Detection | 330 reactions |
| 1-103-0011 | *IGL* Gene Clonality Assay - ABI Fluorescence Detection | 33 reactions |
| 1-103-0021 | *IGL* Gene Clonality Assay MegaKit - ABI Fluorescence Detection | 330 reactions |

# *TCRB* + *TCRG* T-Cell Clonality Assays

## Assay Use

*TCRB* + *TCRG* T-Cell Clonality Assays are useful for studies involving:

- Identification of clonal T-cell populations highly suggestive of T-cell malignancies
- Lineage determination of leukemias and lymphomas
- Monitoring and evaluation of disease recurrence
- Detection and assessment of residual disease
- Evaluation of new research and methods in malignancy studies

## Summary and Explanation of the Test

Five master mixes are included in these test kits to test for rearrangements of both *TRB* (formerly known as *TCRB*) and *TRG* (formerly known as *TCRG*). *TCRB* Tubes A and B target framework regions within the variable region, and the joining region of the T-cell receptor beta locus. *TCRB* Tube C targets the diversity and joining regions. *TCRG* Tubes A and B target framework regions within the variable region, and the joining region of the T-cell receptor gamma locus.

Positive and negative controls, as well as a Specimen Control Size Ladder Master Mix, are included. PCR products can be analyzed by capillary electrophoresis or heteroduplex analysis. Clonality is indicated if any one of the master mixes generates clonal products.

## Background

The human T-cell receptor beta (*TRB*, formerly known as *TCRB*) gene locus on chromosome 7 (7q34, formerly 7q35) includes 64-67 variable (Vβ) gene segments (belonging to 30 subgroups), 2 diversity (Dβ) gene segments, and 13 joining (Jβ) gene segments, spread over 685 kilobases. The diversity of this locus has complicated PCR-based testing, however, this standardized multiplex PCR assay detects the vast majority of clonal *TRB* gene rearrangements using only three multiplex master mixes. The detection rate of clonal *TRB* gene rearrangements using this assay is exceptionally high.[1]

The T-cell receptor gamma (*TRG*, formerly known as *TCRG*) chain locus spans 128 kb on chromosome 7 (7p14). Rearrangement of the variable (Vγ) and joining (Jγ) genetic segments of the *TRG* locus result in Vγ-Jγ products of unique length and sequence. The *TRG* locus does not contain D segments.[2] In addition, the *TRG* gene contains a limited number of Vγ and Jγ segments such that the amplification of all major Vγ-Jγ combinations is possible with four Vγ and two Jγ primers. This standardized multiplex PCR assay detects the vast majority of clonal *TRG* gene rearrangements using two multiplex master mixes.[3] Using this assay, the clonal *TRG* gene rearrangement detection rate is exceptionally high.[3]

PCR products generated from the *TRB* and *TRG* assays are easily and reliably identified by heteroduplex analysis or capillary electrophoresis.

## Specimen Requirements

- 5 mL of peripheral blood, bone marrow biopsy, or bone marrow aspirate anti-coagulated with heparin or EDTA; or,
- Minimum 5 mm cube of tissue; or,
- 3 µg of genomic DNA; or,
- Formalin-fixed, paraffin-embedded (FFPE) tissue or slides.

## Reference

1. M Brüggemann et al., *Leukemia* 17: 2283-2289 (2003).
2. JJM van Dongen et al., *Leukemia* 17: 2257-2317 (2003).
3. K Beldjord et al., *Leukemia* 17: 2289-2292 (2003).

EuroClonality

**This assay is based on the EuroClonality/BIOMED-2 Concerted Action BMH4-CT98-3936.**

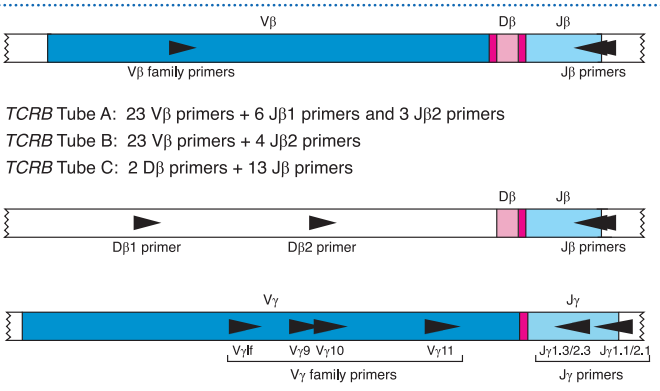



**Figure Legend:** Simplified diagram of a representative rearranged T-cell receptor beta gene and the T-cell receptor gamma gene showing the approximate placement of the upstream and downstream DNA primers. The numbers of primers and their specificity are listed for master mix *TCRB* Tubes A, B, and C and *TCRG* Tubes A and B. (The Vγ1f primer is a consensus primer that targets Vγ1 through Vγ8).

# Gel and Capillary

*TCRB + TCRG* T-Cell Clonality Assays

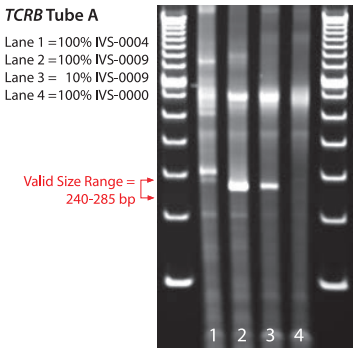
Research Use Only (RUO) Assay

## Reagents

| Controls | Concentration | Units in Assay | Units in Assay MegaKit |
|---|---|---|---|
| IVS-0009 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0004 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0021 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0000 Polyclonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| **Master Mixes** | **Target** | **Units in Assay** | **Units in Assay MegaKit** |
| *TCRB* Tube A | Multiple Vβ + Jβ1/2 | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *TCRB* Tube B | Multiple Vβ + Jβ2 | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *TCRB* Tube C | Multiple Dβ + Jβ1/2 | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *TCRG* Tube A | Vγ1-8 + Vγ10 + Jγ | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *TCRG* Tube B | Vγ9+ Vγ11 + Jγ | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| Specimen Control Size Ladder | Multiple Genes | 1 x 1500 µL tube | 10 x 1500 µL tubes |

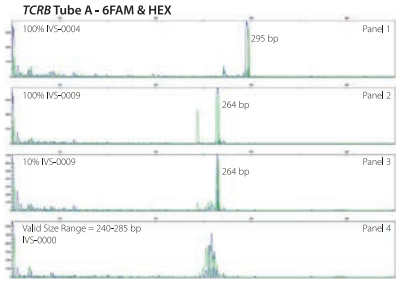
## Gel Electrophoresis Detection

The data shown was generated using the master mix indicated. Amplified products were heteroduplexed and run on a 6% non-denaturing polyacrylamide/TBE gel. Lane 1 is data generated testing an alternative 100% clonal control DNA; lane 2 is data generated testing the recommended 100% clonal control DNA; lane 3 is data generated testing a 10% dilution of the recommended clonal control DNA; and, lane 4 is data generated testing IVS-0000 Polyclonal Control DNA (Cat# 4-092-0010). The IVS-0000 control is used as a negative control for many of our tests. A standard 100 base pair DNA size ladder was run in the lanes flanking the test samples.




## Capillary Electrophoresis Detection (ABI)

The data shown was generated using the master mix indicated. Amplified products were run on an ABI 3100 instrument. Panel 1 displays data generated testing an alternative 100% clonal control DNA; panel 2 displays data generated testing the recommended 100% clonal control DNA; panel 3 displays data generated testing a 10% dilution of the recommended clonal control DNA; and, panel 4 displays data generated testing the IVS-0000 Polyclonal Control DNA (Cat# 4-092-0010). Our IVS-0000 DNA is often tested to provide information regarding valid size ranges for the master mix.




## Ordering Information

| Catalog # | Products | Quantity |
|---|---|---|
| 1-200-0010 | *TCRB + TCRG* T-Cell Clonality Assay - Gel Detection | 33 reactions |
| 1-200-0020 | *TCRB + TCRG* T-Cell Clonality Assay MegaKit - Gel Detection | 330 reactions |
| 1-200-0011 | *TCRB + TCRG* T-Cell Clonality Assay - ABI Fluorescence Detection | 33 reactions |
| 1-200-0021 | *TCRB + TCRG* T-Cell Clonality Assay MegaKit - ABI Fluorescence Detection | 330 reactions |

# *TCRB* Gene Clonality Assays

## Assay Use

*TCRB* Gene Clonality Assays are useful for studies involving:

- Identification clonal T-cell populations highly suggestive of T-cell malignancies
- Lineage determination of leukemias and lymphomas
- Monitoring and evaluation of disease recurrence
- Detection and assessment of residual disease
- Evaluation of new research and methods in malignancy studies

## Summary and Explanation of the Test

Three multiplex master mixes target conserved regions within the variable (Vβ), diversity (Dβ), and the joining (Jβ) regions that flank the unique hypervariable, antigen-binding, complementarity determining region 3 (CDR3) of the T-cell receptor beta locus. Tube A contains 23 Vβ primers, six Jβ1 primers, and three Jβ2 primers. Tube B contains 23 Vβ and four Jβ2 primers. Tube C contains two Dβ and 13 Jβ primers. Positive and negative DNA controls, as well as a Specimen Control Size Ladder Master Mix, are included. PCR products can be analyzed by capillary electrophoresis or heteroduplex analysis. Clonality is indicated, if any one of the master mixes generates clonal products.

## Background

The human T-cell receptor beta (*TRB*, formerly known as *TCRB*) gene locus on chromosome 7 (7q34, formerly 7q35) includes 64-67 variable (Vβ) gene segments (belonging to 30 subgroups), two diversity (Dβ) gene segments, and 13 joining (Jβ) gene segments, spread over 685 kilobases. The diversity of this locus has complicated PCR-based testing and extended dependence on Southern blot analysis in many testing centers. However, this standardized multiplex PCR assay detects the vast majority of clonal *TRB* gene rearrangements using only three multiplex master mixes.[1]

This assay provides rapid *TCR* clonality assessment, reducing or completely eliminating the number of Southern blot tests performed in the laboratory. The detection rate of clonal *TRB* gene rearrangements using this assay is exceptionally high.[1]

## Specimen Requirements

- 5 mL of peripheral blood, bone marrow biopsy, or bone marrow aspirate anti-coagulated with heparin or EDTA; or,
- Minimum 5 mm cube of tissue; or,
- 2 µg of genomic DNA; or,
- Formalin-fixed, paraffin-embedded (FFPE) tissue or slides.

## Reference

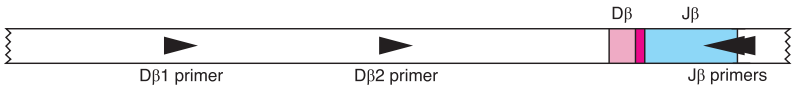
1. M Brüggemann et al., *Leukemia* 17: 2283-2289 (2003).
2. JJM van Dongen et al., *Leukemia* 17: 2257-2317 (2003).

EuroClonality

This assay is based on the **EuroClonality/BIOMED-2** Concerted Action BMH4-CT98-3936.

**Figure Legend:** Simplified diagram of a representative rearranged T-cell receptor beta gene showing the approximate placement of the upstream and downstream DNA primers. The numbers of primers and their specificity are listed for Master Mix Tubes A, B, and C.


Tube A: 23 Vβ primers + 6 Jβ1 primers and 3 Jβ2 primers
Tube B: 23 Vβ primers + 4 Jβ2 primers
Tube C: 2 Dβ primers + 13 Jβ primers



# Gel and Capillary

*TCRB* Gene Clonality Assays

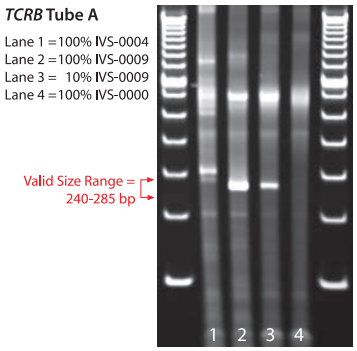
Research Use Only (RUO) Assay

## Reagents

| Controls | Concentration | Units in Assay | Units in Assay MegaKit |
|---|---|---|---|
| IVS-0009 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0004 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0021 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0000 Polyclonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| **Master Mixes** | **Target** | **Units in Assay** | **Units in Assay MegaKit** |
| *TCRB* Tube A | Multiple Vβ + Jβ1/2 | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *TCRB* Tube B | Multiple Vβ + Jβ2 | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *TCRB* Tube C | Multiple Dβ + Jβ1/2 | 1 x 1500 µL tube | 10 x 1500 µL tubes |

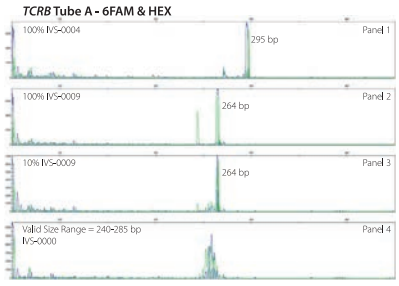
## Gel Electrophoresis Detection

The data shown was generated using the master mix indicated. Amplified products were heteroduplexed and run on a 6% non-denaturing polyacrylamide/TBE gel. Lane 1 is data generated testing an alternative 100% clonal control DNA; lane 2 is data generated testing the recommended 100% clonal control DNA; lane 3 is data generated testing a 10% dilution of the recommended clonal control DNA; and, lane 4 is data generated testing IVS-0000 Polyclonal Control DNA (Cat# 4-092-0010). The IVS-0000 control is used as a negative control for many of our tests. A standard 100 base pair DNA size ladder was run in the lanes flanking the test samples.




## Capillary Electrophoresis Detection (ABI)

The data shown was generated using the master mix indicated. Amplified products were run on an ABI 3100 instrument. Panel 1 displays data generated testing an alternative 100% clonal control DNA; panel 2 displays data generated testing the recommended 100% clonal control DNA; panel 3 displays data generated testing a 10% dilution of the recommended clonal control DNA; and, panel 4 displays data generated testing the IVS-0000 Polyclonal Control DNA (Cat# 4-092-0010). Our IVS-0000 DNA is often tested to provide information regarding valid size ranges for each master mix.




## Ordering Information

| Catalog # | Products | Quantity |
|---|---|---|
| 1-205-0010 | *TCRB* Gene Clonality Assay - Gel Detection | 33 reactions |
| 1-205-0020 | *TCRB* Gene Clonality Assay MegaKit - Gel Detection | 330 reactions |
| 1-205-0011 | *TCRB* Gene Clonality Assay - ABI Fluorescence Detection | 33 reactions |
| 1-205-0021 | *TCRB* Gene Clonality Assay MegaKit - ABI Fluorescence Detection | 330 reactions |

# T-Cell Receptor Gamma Gene Rearrangement Assay 2.0

## Assay Use

T-Cell Receptor Gamma Gene Rearrangement Assays are useful for studies involving:

- Identification of clonal T-cell populations highly suggestive of T-cell malignancies
- Monitoring and evaluation of disease recurrence
- Evaluation of new research and methods in malignancy studies

## Summary and Explanation of the Test

This T-Cell Receptor Gamma Gene Rearrangement Assay 2.0 represents an improved approach to PCR-based clonality testing of lymphoproliferative disorders, as it can detect the vast majority of *TCR* gamma gene rearrangements with a single multiplex master mix. Importantly, this assay includes, in a single tube, primers for all known groups of *TCR* gamma variable (Vγ) region genes and joining (Jγ) region genes that are involved in rearrangements of T-cell lymphomas. In addition, all reverse primers that target the Jγ region genes are conjugated with the 6FAM fluorophore. Positive and negative controls, as well as a Specimen Control Size Ladder Master Mix are included. PCR products are analyzed by capillary electrophoresis.

## Background

The human T-cell receptor gamma (*TRG*, formerly known as *TCRG*) gene locus on chromosome 7 (7q14) includes 14 Vγ genes belonging to four subgroups, five Jγ segments, and two Cγ genes spread over 200 kilobases. The diversity of this locus has historically complicated PCR-based testing. Our new multiplex PCR assay represents an improvement over existing assays as it can detect the vast majority of *TCR* gamma gene rearrangements with a single multiplex master mix. This master mix targets all conserved regions within the variable (Vγ) and joining (Jγ) region genes that are described in lymphoid malignancies. This is critical for more comprehensive analysis of patient samples, as some T-cell lymphoproliferative disorders involve Vγ and Jγ regions that would not be identified with a single Vγ(1-8) and Jγ1/Jγ2 primer set.

In addition, the polyclonal background that results from the combination of all primers in a single tube produces a more robust and easily interpreted signal with capillary electrophoresis, which aids in the interpretation of small peaks. Competitive amplification of all *TRG* gene rearrangements allows for identification of a quantitative threshold for a positive result and helps to avoid false positive results. The average size of the *TRG* gene rearrangement PCR amplicons is 190 nucleotides, with a normal distribution of product sizes between 159 and 207 nucleotides. This protocol should lead to improved product formation from formalin-fixed, paraffin-embedded (FFPE) samples compared to other protocols that yield products of 260 nucleotides or larger.

## Specimen Requirements

- 5 mL of peripheral blood, bone marrow biopsy, or bone marrow aspirate anti-coagulated with heparin or EDTA; or,
- Minimum 5 mm cube of tissue; or,
- 2 µg of genomic DNA; or,
- Formalin-fixed, paraffin-embedded tissue or slides.

## Reference

1. TC Greiner et al., *JMD* 4: 137-143 (2002).
2. LC Lawnickie et al., *JMD* 5: 82-87 (2003).
3. Y Sandberg et al., *Leukemia* 21: 21 (2007).
4. Armand, Marine et al. *HemaSphere*, 2019;3:3.

EuroClonality **This assay was developed by Invivoscribe.**
**The performance of this assay was reviewed and validated by the EuroClonality/BIOMED-2 Group.**[4]

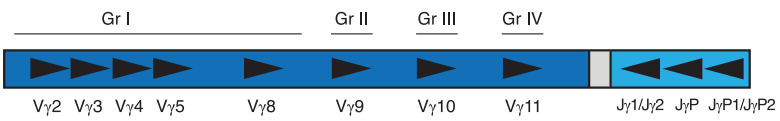



**Figure Legend:** Simple representation of the organization of the T-cell receptor gamma gene on chromosome 7. Black arrows represent the relative positions of primers that target the variable region genes and the downstream joining region gene segments that are involved in rearrangements in T-cell lymphomas. The downstream primers are fluorescently labeled through the incorporation of a 6FAM fluorophore. The amplicon products generated from these rearrangements are detected by capillary electrophoresis.

# Gel and Capillary

T-Cell Receptor Gamma Gene Rearrangement Assay 2.0

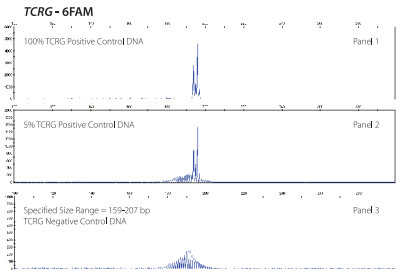
Research Use Only (RUO) Assay

## Reagents

| Controls | Concentration | Units in Assay | Units in Assay MegaKit |
|---|---|---|---|
| 5% *TCRG* Positive Control DNA | 50 µg/mL | 1 x 50 µL tube | 5 x 50 µL tube |
| *TCRG* Negative Control DNA | 50 µg/mL | 1 x 50 µL tube | 5 x 50 µL tube |
| **Master Mixes** | **Target** | **Units in Assay** | **Units in Assay MegaKit** |
| *TCRG* - 6FAM | Vγ1-Vγ11 + Jγ1/Jγ2, JγP, JγP1/JγP2 | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| Specimen Control Size Ladder | Multiple Genes | 1 x 1500 µL tube | 10 x 1500 µL tubes |

## Capillary Electrophoresis Detection (ABI)

The data shown was generated using the *TCRG*-6FAM master mix. Amplified products were run on a capillary electrophoresis ABI 3130xl instrument. Panel 1 displays data generated testing 100% *TCRG* Positive Control DNA (DNA isolated from a cell line known to have both a Vγ9 + Jγ1/Jγ2 and a Vγ10 + Jγ1/Jγ2 rearrangement); panel 2 displays data generated from the testing of the 5% *TCRG* Positive Control DNA; and, panel 3 displays data generated from the testing of the polyclonal *TCRG* Negative Control DNA.




## Ordering Information

| Catalog # | Products | Quantity |
|---|---|---|
| 1-207-0101 | T-Cell Receptor Gamma Gene Rearrangement Assay 2.0 - ABI Fluorescence Detection | 33 reactions |
| 1-207-0111 | T-Cell Receptor Gamma Gene Rearrangement Assay MegaKit 2.0 - ABI Fluorescence Detection | 330 reactions |

# T-Cell Receptor Gamma Gene Rearrangement Assays

## Assay Use

T-Cell Receptor Gamma Gene Rearrangement Assays are useful for studies involving:

- Identification of clonal T-cell populations highly suggestive of T-cell malignancies
- Lineage determination of leukemias and lymphomas
- Monitoring and evaluation of disease recurrence
- Detection and assessment of residual disease
- Evaluation of new research and methods in malignancy studies

## Summary and Explanation of the Test

Sample genomic DNA is amplified using two master mixes that independently target conserved regions within the variable (Vγ) and joining (Jγ) regions that flank the unique, hypervariable, antigen-binding, complementarity determining region 3 (CDR3). This assay targets Vγ1-9 and Jγ gene segments. Positive and negative DNA controls, as well as an internal Amplification Control Master Mix, are included. The limit of detection of this assay is approximately one clonal T cell in a background of a hundred normal cells. PCR products can be analyzed by capillary electrophoresis or standard gel electrophoresis with ethidium bromide staining.

## Background

The T-cell receptor gamma (*TRG*, formerly known as *TCRG*) chain locus spans 160 kilobases on chromosome 7 (7p14). The locus consists of 14 variable (Vγ) gene segments in six subgroups, and five joining (Jγ) gene segments interspersed between two constant (Cγ) gene segments. However, the repertoire of functional TRG molecules is limited to 4-6 functional Vγ gene segments that belong to two subgroups.[2]

Rearrangement of the Vγ and Jγ gene segments of the *TRG* locus results in Vγ-Jγ products of unique length and sequence. Clonal *TRG* rearrangements can be most rapidly identified by analyzing the size distribution of DNA products amplified from conserved sequences that flank this Vγ-Jγ region.[1] DNA isolated from a normal heterogeneous population of polyclonal T-cells produces a Gaussian distribution (bell-shaped size curve) of amplified products. DNA amplified from a clonal T-cell population generates one or two product(s) of unique size that reflects proliferation of a single rearranged clone.[1,2]

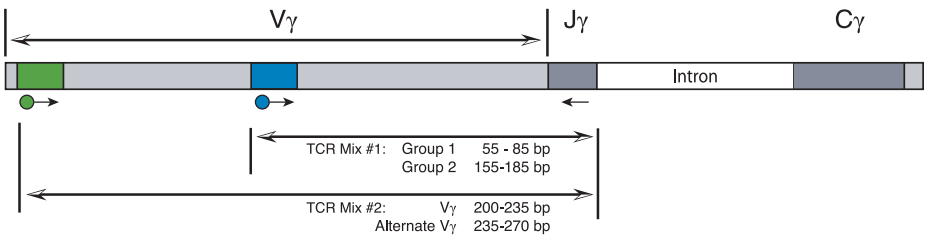
## Specimen Requirements

- 5 mL of peripheral blood, bone marrow biopsy, or bone marrow aspirate anti-coagulated with heparin or EDTA; or,
- Minimum 5 mm cube of tissue; or,
- 2 µg of genomic DNA; or,
- Formalin-fixed, paraffin-embedded (FFPE) tissue or slides.

## Reference

1. JE Miller, SS Wilson, DL Jaye, and M Kronenberg. *J. Mol. Diag.* 4: 101-117 (1999).
2. K Beldjord et al., *Leukemia* 17: 2289-2292 (2003).

**Figure Legend:** Simplified figure representing the organization of a rearranged T-cell receptor gamma chain gene on chromosome 7. Colored arrows represent conserved regions within the variable region gene segments targeted by primers. Primers are represented by arrows with the size range of valid products generated with each of the master mixes indicated below the figure. Colors correspond to the peak colors assigned to products when differential fluorescence detection methods are used.



# Gel and Capillary

T-Cell Receptor Gamma Gene Rearrangement Assays

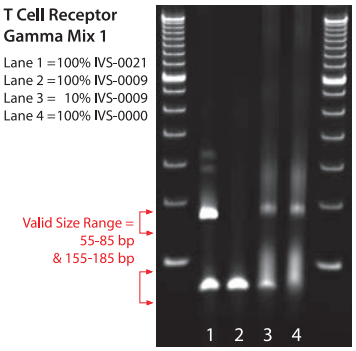
Research Use Only (RUO) Assay

## Reagents

| Controls | Concentration | Units in Assay | Units in Assay MegaKit |
|---|---|---|---|
| IVS-0009 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0000 Polyclonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| **Master Mixes** | **Target** | **Units in Assay** | **Units in Assay MegaKit** |
| T-Cell Receptor Gamma Mix 1 | Vγ1-8,9 + Jγ1/2 | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| T-Cell Receptor Gamma Mix 2 | Alt Vγ + Jγ1/2 | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| Amplification Control | *HLA-DQa* | 1 x 1500 µL tube | 10 x 1500 µL tubes |

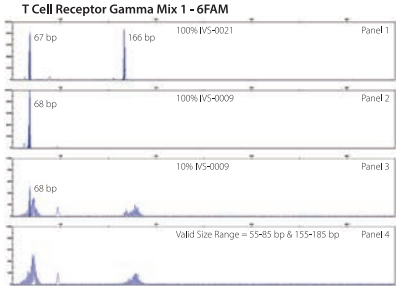
## Gel Electrophoresis Detection

The data shown was generated using the master mix indicated. Amplified products were run on a 6% non-denaturing polyacrylamide/TBE gel. Lane 1 is data generated testing an alternative 100% clonal control DNA; lane 2 is data generated testing the recommended 100% clonal control DNA; lane 3 is data generated testing a 10% dilution of the recommended clonal control DNA; and, lane 4 is data generated testing IVS-0000 Polyclonal Control DNA (Cat# 4-092-0010). The IVS-0000 control is used as a negative control for many of our tests. A standard 100 base pair DNA size ladder was run in the lanes flanking the test samples.




## Capillary Electrophoresis Detection (ABI)

The data shown was generated using the master mix indicated. Amplified products were run on an ABI 3100 instrument. Panel 1 displays data generated testing an alternative 100% clonal control DNA; panel 2 displays data generated testing the recommended 100% clonal control DNA; panel 3 displays data generated testing a 10% dilution of the recommended clonal control DNA; and, panel 4 displays data generated testing the IVS-0000 Polyclonal Control DNA (Cat# 4-092-0010). Our IVS-0000 DNA is often tested to provide information regarding valid size ranges for each master mix.




## Ordering Information

| Catalog # | Products | Quantity |
|---|---|---|
| 1-207-0010 | T-Cell Receptor Gene Rearrangement Assay - Gel Detection | 30 reactions |
| 1-207-0030 | T-Cell Receptor Gene Rearrangement Assay MegaKit - Gel Detection | 300 reactions |
| 1-207-0051 | T-Cell Receptor Gene Rearrangement Assay - ABI Fluorescence Detection | 30 reactions |
| 1-207-0071 | T-Cell Receptor Gene Rearrangement Assay MegaKit - ABI Fluorescence Detection | 300 reactions |

Research Use Only (RUO) Assay

# *TCRG* Gene Clonality Assays

## Assay Use

*TRG* Gene Clonality Assays are useful for studies involving:

- Identification of clonal T-cell populations highly suggestive of T-cell malignancies
- Lineage determination of leukemias and lymphomas
- Monitoring and evaluation of disease recurrence
- Detection and assessment of residual disease
- Evaluation of new research and methods in malignancy studies

## Summary and Explanation of the Test

This assay tests all variable (Vγ) regions 1-11 of the *TRG* (formerly known as *TCRG*) gene.. Master mix tubes A and B target conserved regions within the variable (Vγ) and joining (Jγ) regions that flank the unique, hypervariable, antigen-binding, complementarity determining region 3 (CDR3). Tube A contains two Vγ primers and two Jγ primers. Tube B contains two Vγ primers and two Jγ primers. Positive and negative controls, as well as a Specimen Control Size Ladder Master Mix are included. PCR products can be analyzed by capillary electrophoresis or heteroduplex analysis. Clonality is indicated if any one of the master mixes generates clonal products.

## Background

The T-cell receptor gamma (*TRG*, formerly known as *TCRG*) chain locus spans 128 kb on chromosome 7 (7p14). Rearrangement of the variable (Vγ) and joining (Jγ) genetic segments of the *TRG* locus result in Vγ-Jγ products of unique length and sequence. The *TRG* locus does not contain diversity (Dγ) segments.[2] *TRG* is a preferential target for clonality analyses since it is rearranged in greater than 90% of T-ALL, T-large granular lymphocyte (LGL), and T-PLL, in 50-75% of peripheral T-NHL and mycosis fungoides, but not NK cell proliferations. It is also rearranged in a major part (60%) of B-lineage ALLs and in a much smaller part of B NHLs. In addition, the *TRG* gene contains a limited number of Vγ and Jγ segments such that the amplification of all major Vγ-Jγ combinations is possible with four Vγ and two Jγ primers.

This standardized multiplex PCR assay detects the vast majority of clonal *TRG* gene rearrangements using only two multiplex master mixes.[1] The potential risk of false-positive results, due to overinterpretation of minor clonal peaks, can be minimized by the combined use of heteroduplex analysis and differential fluorescence detection, and by interpreting results within their clinical context.[1,2]

The detection rate of clonal *TRG* gene rearrangements using this assay is exceptionally high.[1]

## Specimen Requirements

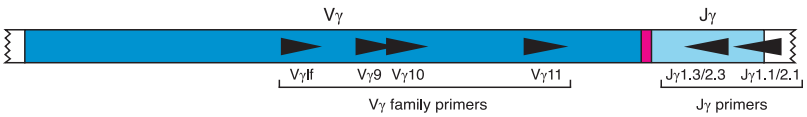
- 5 mL of peripheral blood, bone marrow biopsy, or bone marrow aspirate anti-coagulated with heparin or EDTA; or,
- Minimum 5 mm cube of tissue; or,
- 2 µg of genomic DNA; or,
- Formalin-fixed, paraffin-embedded (FFPE) tissue or slides.

## Reference

1. K Beldjord et al., *Leukemia* 17: 2289-2292 (2003).
2. JJM van Dongen et al., *Leukemia* 17: 2257-2317 (2003).

EuroClonality **This assay is based on the EuroClonality/BIOMED-2 Concerted Action BMH4-CT98-3936.**

**Figure Legend:** Depicted is a simple representation of the organization of the T-cell receptor gamma chain gene on chromosome 7. Black arrows represent the relative positions of primers that target the variable (Vγ) regions, and the downstream joining (Jγ) gene segments. The amplicon products generated from each of these regions can be differentially detected when fluorescent primer sets are used with capillary electrophoresis instruments that employ differential fluorescence detection.




*TCRG* tube A: Vγlf and Vγ10 primers + Jγ1.1/2.1 and Jγ1.3/2.3

*TCRG* tube B: Vγ9 and Vγ11 primers + Jγ1.1/2.1 and Jγ1.3/2.3

# Gel and Capillary

*TCRG* Gene Clonality Assays

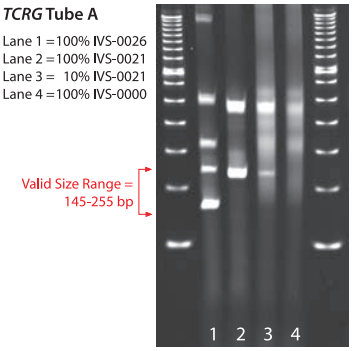
Research Use Only (RUO) Assay

## Reagents

| Controls | Concentration | Units in Assay | Units in Assay MegaKit |
|---|---|---|---|
| IVS-0009 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0021 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0000 Polyclonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| **Master Mixes** | **Target** | **Units in Assay** | **Units in Assay MegaKit** |
| *TCRG* Tube A | Vγ1-8 + Vγ10 + Jγ | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *TCRG* Tube B | Vγ9 + Vγ11 + Jγ | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| Specimen Control Size Ladder | Multiple Genes | 1 x 1500 µL tube | 10 x 1500 µL tubes |

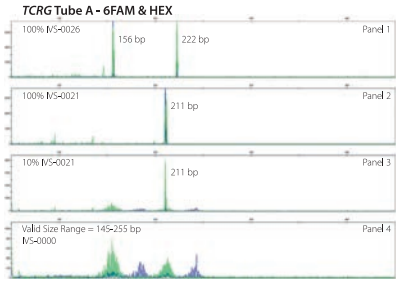
## Gel Electrophoresis Detection

The data shown was generated using the master mix indicated. Amplified products were heteroduplexed and run on a 6% non-denaturing polyacrylamide/TBE gel. Lane 1 is data generated testing an alternative 100% clonal control DNA; lane 2 is data generated testing the recommended 100% clonal control DNA; lane 3 is data generated testing a 10% dilution of the recommended clonal control DNA; and, lane 4 is data generated testing IVS-0000 Polyclonal Control DNA (Cat# 4-092-0010). The IVS-0000 control is used as a negative control for many of our tests. A standard 100 base pair DNA size ladder was run in the lanes flanking the test samples.




## Capillary Electrophoresis Detection (ABI)

The data shown was generated using the master mix indicated. Amplified products were run on an ABI 3100 instrument. Panel 1 displays data generated testing an alternative 100% clonal control DNA; panel 2 displays data generated testing the recommended 100% clonal control DNA; panel 3 displays data generated testing a 10% dilution of the recommended clonal control DNA; and, panel 4 displays data generated testing the IVS-0000 Polyclonal Control DNA (Cat# 4-092-0010). Our IVS-0000 DNA is often tested to provide information regarding valid size ranges for each master mix.




## Ordering Information

| Catalog # | Products | Quantity |
|---|---|---|
| 1-207-0020 | *TRG* Gene Clonality Assay - Gel Detection | 33 reactions |
| 1-207-0040 | *TRG* Gene Clonality Assay MegaKit - Gel Detection | 330 reactions |
| 1-207-0021 | *TRG* Gene Clonality Assay - ABI Fluorescence Detection | 33 reactions |
| 1-207-0041 | *TRG* Gene Clonality Assay MegaKit - ABI Fluorescence Detection | 330 reactions |

# *TCRD* Gene Clonality Assays

## Assay Use

*TCRD* Gene Clonality Assays are useful for studies involving:

- Identification of clonal T-cell populations highly suggestive of T-cell malignancies
- Lineage determination of leukemias and lymphomas
- Monitoring and evaluation of disease recurrence
- Detection and assessment of residual disease
- Evaluation of new research and methods in malignancy studies

## Summary and Explanation of the Test

The *TCRD* Tube Master Mix targets conserved regions within the variable (Vδ1-6), the diversity (Dδ2-3) and the joining (Jδ1-4) regions that flank the unique, hypervariable, antigen-binding, complementarity determining region 3 (CDR3) of the T-cell receptor delta (*TRD*, formerly known as *TCRD*). Positive and negative controls, as well as a Specimen Control Size Ladder Master Mix, are included. PCR products can be analyzed by capillary electrophoresis or heteroduplex analysis. Clonality is indicated if the master mix generates clonal products.

## Background

The human T-cell receptor delta (*TRD*, formerly known as *TCRD*) gene locus is comprised of a cluster of 10 genes located on chromosome 14 (14q11.2) spread over 60 kilobases, localized between the T-cell receptor alpha (*TRA*, formerly known as *TCRA*) variable (Vα) and joining (Jα) gene segments. It is made up of eight variable (Vδ), three diversity (Dδ), and four joining (Jδ) gene segments.[1] At least five of the eight Vδ gene segments can also rearrange to Jδ gene segments and other Vδ gene segments may also be utilized in *TRD* gene rearrangements in rare cases. Although the small number of Vδ, Dδ, and Jδ gene segments available for recombination limits the potential combinatorial diversity, the complementarity determining region 3 (CDR3) or junctional diversity is extensive due to the addition of N regions, P regions, and random deletion of nucleotides by recombinases. This diversity is also extended by the recombination of up to three Dδ segments and therefore up to four N regions within the rearranged *TRD* locus. This limited germline diversity encoded at the *TRD* locus in conjunction with extensive junctional diversity results in a useful target for PCR analysis. *TRD* recombination events have been used most extensively as clonal markers in both T- and B-cell ALL. This standardized multiplex PCR assay detects the vast majority of clonal *TRD* gene rearrangements using a single multiplex master mix[1]. This assay provides rapid *TCR* clonality assessment, reducing or eliminating the number of Southern blot tests performed in the laboratory.[2] The detection rate of clonal *TRD* gene rearrangements using this assay is exceptionally high.[1]

## Specimen Requirements

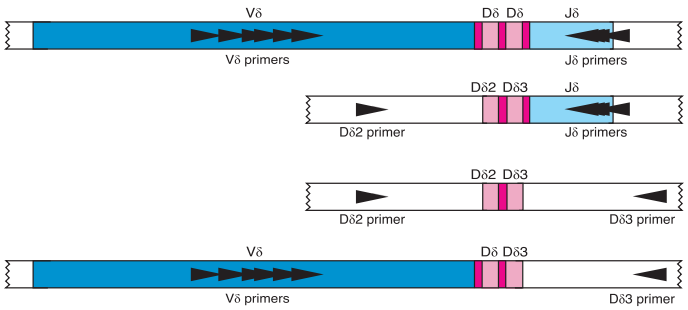
- 5 mL of peripheral blood, bone marrow biopsy, or bone marrow aspirate anti-coagulated with heparin or EDTA; or,
- Minimum 5 mm cube of tissue; or,
- 2 µg of genomic DNA; or,
- Formalin-fixed, paraffin-embedded (FFPE) tissue or slides.

## Reference

1. FL Lavender et al., *Leukemia* 17: 2292-2296 (2003).
2. JJM van Dongen et al., *Leukemia* 17: 2257-2317 (2003).

EuroClonality This assay is based on the **EuroClonality/BIOMED-2** Concerted Action BMH4-CT98-3936.

**Figure Legend:** Simplified diagram of a representative rearranged T-cell receptor delta gene showing the approximate placement of the upstream and downstream DNA primers. The numbers of primers and their specificity are listed for the *TRD* Tube Master Mix tube.




*TCRD* tube: 6 Vδ and 1 Dδ2 primers + 4 Jδ and 1 Dδ3 primers

# Gel and Capillary

*TCRD* Gene Clonality Assays

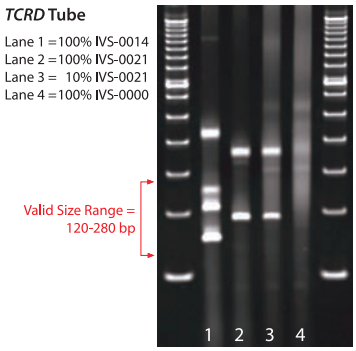
Research Use Only (RUO) Assay

## Reagents

| Controls | Concentration | Units in Assay | Units in Assay MegaKit |
|---|---|---|---|
| IVS-0021 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0000 Polyclonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| **Master Mixes** | **Target** | **Units in Assay** | **Units in Assay MegaKit** |
| *TCRD* Tube | Multiple Vδ + Dδ + Jδ | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| Specimen Control Size Ladder | Multiple Genes | 1 x 1500 µL tube | 10 x 1500 µL tubes |

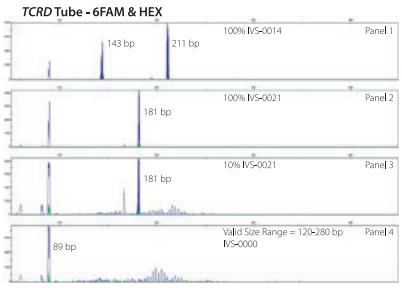
## Gel Electrophoresis Detection

The data shown was generated using the master mix indicated. Amplified products were heteroduplexed and run on a 6% non-denaturing polyacrylamide/TBE gel. Lane 1 is data generated testing an alternative 100% clonal control DNA; lane 2 is data generated testing the recommended 100% clonal control DNA; lane 3 is data generated testing a 10% dilution of the recommended clonal control DNA; and, lane 4 is data generated testing IVS-0000 Polyclonal Control DNA (Cat# 4-092-0010). The IVS-0000 control is used as a negative control for many of our tests. A standard 100 base pair DNA size ladder was run in the lanes flanking the test samples.




## Capillary Electrophoresis Detection (ABI)

The data shown was generated using the master mix indicated. Amplified products were run on an ABI 3100 instrument. Panel 1 displays data generated testing an alternative 100% clonal control DNA; panel 2 displays data generated testing the recommended 100% clonal control DNA; panel 3 displays data generated testing a 10% dilution of the recommended clonal control DNA; and, panel 4 displays data generated testing the IVS-0000 Polyclonal Control DNA (Cat# 4-092-0010). Our IVS-0000 DNA is often tested to provide information regarding valid size ranges for each master mix.




## Ordering Information

| Catalog # | Products | Quantity |
|---|---|---|
| 1-206-0010 | *TCRD* Gene Clonality Assay - Gel Detection | 33 reactions |
| 1-206-0020 | *TCRD* Gene Clonality Assay MegaKit - Gel Detection | 330 reactions |
| 1-206-0011 | *TCRD* Gene Clonality Assay - ABI Fluorescence Detection | 33 reactions |
| 1-206-0021 | *TCRD* Gene Clonality Assay MegaKit - ABI Fluorescence Detection | 330 reactions |

# *BCL1/*JH Translocation Assay

## Assay Use

The *BCL1/*JH Translocation Assay is useful for studies involving:

- Identification of *IGH-BCL1* (now known as *IGH-CCND1*) gene rearrangements highly suggestive of mantle cell lymphoma
- Lineage determination of leukemias and lymphomas
- Monitoring and evaluation of disease recurrence
- Detection and assessment of residual disease
- Evaluation of new research and methods in malignancy studies

## Summary and Explanation of the Test

Two master mixes are included in this assay kit. The *BCL1/*JH Master Mix targets the major translocation cluster (MTC) of the *CCND1* locus (formerly known as *BCL1*) and the joining region (JH) of the immunoglobulin heavy chain locus (IGH). The Specimen Control Size Ladder Master Mix targets multiple genes and generates a series of amplicons of approximately 100, 200, 300, 400, and 600 base pairs to ensure that the quality and quantity of input DNA is adequate to yield a valid result. Positive and negative controls are included. PCR products can be analyzed using standard gel electrophoresis with ethidium bromide staining. A *CCND1* translocation is indicated if the master mix generates product(s) within the valid size range.

## Background

The *IGH-CCND1* t(11;14)(q13;q32) translocation is mainly found in mantle cell lymphomas, but has also been seen in B-prolymphocytic leukemia (10-20%), plasma cell leukemia, splenic lymphoma with villous lymphocytes, chronic lymphocytic leukemia (2-5%), and in multiple myeloma (20-25%). The t(11;14) brings about juxtaposition of the cyclin D1 gene with the immunoglobulin heavy chain gene. This leads to a marked increase in expression of cyclin D1 driven by the Ig heavy chain gene enhancer, located in the intron between the JH and constant region genes. The overexpression of cyclin D1 accelerates the passage of transformed cells through the G1 phase. Approximately 41% of the breakpoints on the *CCND1/MTC* locus can be detected by PCR methodology. However, breakpoints outside of the *CCND1/MTC* locus will not be identified by this particular test. Therefore, a negative result does not completely exclude the presence of an *IGH-CCND1* gene translocation in the sample.[1]

## Specimen Requirements

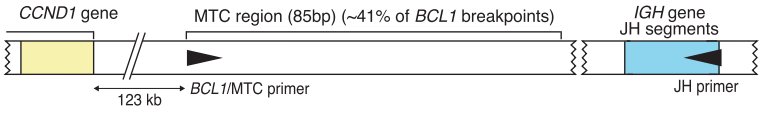
- 5 mL of peripheral blood, bone marrow biopsy, or bone marrow aspirate anti-coagulated with heparin or EDTA; or,
- Minimum 5 mm cube of tissue; or,
- 2 µg of genomic DNA; or,
- Formalin-fixed, paraffin-embedded (FFPE) tissue or slides.

## Reference

1. P Wijers et al., *Leukemia* 17: 2296-2298 (2003).
2. JJM van Dongen et al., *Leukemia* 17: 2257-2317 (2003).

EuroClonality **This assay is based on the EuroClonality/BIOMED-2 Concerted Action BMH4-CT98-3936.**

**Figure Legend:** Schematic diagram of the *IGH-CCND1* t(11;14) translocation showing the cyclin D1 (*CCND1*) gene on the left and the Ig heavy chain (*IGH*) gene on the right. Shown are the relative positions and orientations for the *BCL1/*MTC primer and the JH primer, which are included in the *BCL1/*JH Master Mix tube.




t(11;14) tube: 1 *BCL1* MTC primer + 1 JH primer

# Gel and Capillary

*BCL1/*JH Translocation Assay

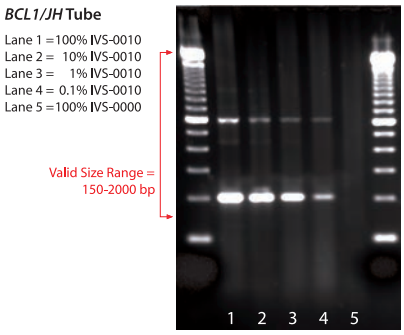
Research Use Only (RUO) Assay

## Reagents

| Controls | Concentration | Units in Assay | Units in Assay MegaKit |
|---|---|---|---|
| IVS-0010 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0000 Polyclonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| **Master Mixes** | **Target** | **Units in Assay** | **Units in Assay MegaKit** |
| *BCL1/*JH Tube | MTC of *CCND1* + *IGH* JH | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| Specimen Control Size Ladder | Multiple Genes | 1 x 1500 µL tube | 10 x 1500 µL tubes |

## Agarose Gel Electrophoresis Detection

The data shown was generated using the master mix indicated. Amplified products were run on a 2% agarose/TBE gel. Lane 1 is data generated testing the recommended 100% clonal control DNA; lane 2 is data generated testing a 10% dilution of the recommended clonal control DNA; lane 3 is data generated testing a 1% dilution of the recommended clonal control DNA; lane 4 is data generated testing a 0.1% dilution of the recommended clonal control DNA; and, lane 5 is data generated testing IVS-0000 Polyclonal Control DNA (Cat# 4-092-0010). This control is used as a negative control for many of our tests. A standard 100 base pair DNA size ladder was run in the lanes flanking the test samples.




## Ordering Information

| Catalog # | Products | Quantity |
|---|---|---|
| 1-308-0010 | *BCL1/*JH Translocation Assay - Gel Detection | 33 reactions |
| 1-308-0020 | *BCL1/*JH Translocation Assay MegaKit - Gel Detection | 330 reactions |

# *BCL2/*JH t(14;18) Translocation Assay

## Assay Use

The *BCL2/*JH t(14;18) Translocation Assay is useful for studies involving:

- Monitoring and evaluation of follicular lymphomas and other B-cell lymphomas
- Distinguishing lymphoma from benign lymphoid hyperplasia
- Distinguishing follicular lymphoma from other B-cell lymphomas that may have a similar appearance
- Monitoring and evaluation of disease recurrence
- Detection and assessment of residual disease
- Evaluation of new research and methods in malignancy studies

## Summary and Explanation of the Test

Five master mixes are included in this assay kit. Two master mixes target *BCL2* major break point (MBR) translocations and two target *BCL2* minor cluster region (mcr) translocations. An Amplification Control Master Mix is also included to ensure the quality and quantity of sample DNA. Positive and negative controls are also included. This assay can be run either in a standard or nested assay format.

Using the standard method, the limit of detection is one cell in one hundred normal cells. The nested method has a limit of detection of one t(14;18) positive cell in a background of ten thousand normal cells. PCR products can be analyzed by standard gel electrophoresis with ethidium bromide staining. A *BCL2* translocation is indicated if just one of the 2nd round master mixes (mixes ending in b) generates product(s) within the valid size range.

## Background

The *IGH-BCL2* t(14;18)(q32;q21) translocation is found in 80-90% of follicular lymphomas and in 30% of diffuse large cell lymphomas.[1,2] The translocation is rarely present in other lymphoproliferative diseases.[2] The t(14;18) brings about juxtaposition of *BCL2* with the Ig heavy chain joining segment. This leads to a marked increase in expression of *BCL2* driven by the Ig heavy chain gene enhancer.[1] The *BCL2* protein inhibits programmed cell death (apoptosis) leading to cell accumulation.[2] The majority of breakpoints on 18q21-22 occur within the major breakpoint region (MBR) in the 3′ untranslated region of exon 3 (60-70% of the cases), and the minor cluster region (mcr) located 3′ to *BCL2* exon 3 (20-25% of the cases).[1] Some breakpoints occur at distant loci and will not be identified by this particular test.[2] Therefore, a negative result does not completely exclude the presence of a *IGH-BCL2* gene rearrangement in the sample. In comparison, Southern blot analysis requires 1-2 weeks, is significantly less sensitive, and has more restrictive Specimen requirements.

## Specimen Requirements

- 5 mL of peripheral blood, bone marrow biopsy, or bone marrow aspirate anti-coagulated with heparin or EDTA; or,
- Minimum 5 mm cube of tissue; or,
- 3 µg of genomic DNA; or,
- Formalin-fixed, paraffin-embedded (FFPE) tissue or slides.

## Reference

1. MS Lee et al., *Science* 237: 175-178 (1987).
2. M Crescenzi et al., *Proc. Natl. Acad. Sci. USA* 85: 4869-4873 (1988).

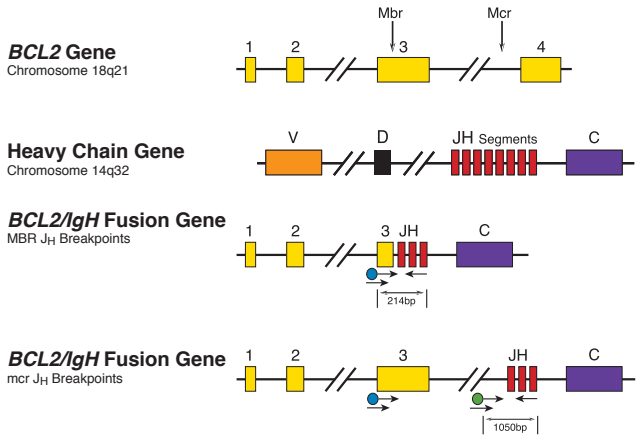



**Figure Legend:** Simplified view of the genomic organization of the *BCL2* and *IGH* genes on chromosomes 18 and 14, respectively. Yellow boxes represent the exon regions of the *BCL2* gene. Exons of the immunoglobulin heavy chain gene are represented in other colors. The solid black lines represents intron regions, which have been left incompletely spliced to assist in demarcation of the exon segments. MBR and mcr type t(14;18) translocations are shown in the lower portions of the figure with the relative positions of primers and the size of the amplicons generated from the positive control DNAs indicated.

# Gel and Capillary

*BCL2/*JH t(14;18) Translocation Assay

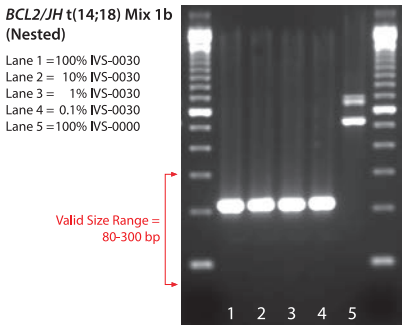
Research Use Only (RUO) Assay

## Reagents

| Controls | Concentration | Units in Assay | Units in Assay MegaKit |
|---|---|---|---|
| IVS-0030 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0031 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0009 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| **Master Mixes** | **Target** | **Units in Assay** | **Units in Assay MegaKit** |
| *BCL2/*JH t(14;18) (MBR) Mix 1b | Inside *BCL2* MBR | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *BCL2/*JH t(14;18) (mcr) Mix 2b | Inside *BCL2* mcr | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *BCL2/*JH t(14;18) (MBR) Mix 1a | Outside *BCL2* MBR | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *BCL2/*JH t(14;18) (mcr) Mix 2a | Outside *BCL2* mcr | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| Amplification Control | *HLA-DQa* | 1 x 1500 µL tube | 10 x 1500 µL tubes |

## Gel Electrophoresis Detection

The data shown was generated using the master mix indicated. Amplified products were run on a 2% agarose/TBE gel. Lane 1 is data generated testing the recommended 100% clonal control DNA; lane 2 is data generated testing a 10% dilution of the recommended clonal control DNA; lane 3 is data generated testing a 1% dilution of the recommended clonal control DNA; lane 4 is data generated testing a 0.1% dilution of the recommended clonal control DNA; and, lane 5 is data generated testing IVS-0000 Polyclonal Control DNA (Cat# 4-092-0010). The IVS-0000 control is used as a negative control for many of our tests. A standard 100 base pairs DNA size ladder was run in the lanes flanking the test samples.




## Ordering Information

| Catalog # | Products | Quantity |
|---|---|---|
| 1-309-0010 | *BCL2/*JH t(14;18) Translocation Assay - Gel Detection | 30 reactions |
| 1-309-0030 | *BCL2/*JH t(14;18) Translocation Assay MegaKit - Gel Detection | 300 reactions |

# *BCL2/*JH Translocation Assay

## Assay Use

The *BCL2/*JH Translocation Assay is useful for studies involving:

- Monitoring and evaluation of follicular lymphomas and other B-cell lymphomas
- Distinguishing lymphoma from benign lymphoid hyperplasia
- Distinguishing follicular lymphoma from other B-cell lymphomas that may have a similar appearance
- Monitoring and evaluation of disease recurrence
- Detection and assessment of residual disease
- Evaluation of new research and methods in malignancy studies

## Summary and Explanation of the Test

Four master mixes are included in this assay. Three are used to identify translocations in the major breakpoint region (MBR) and minor cluster region (mcr) of *BCL2*. The Specimen Control Size Ladder master mix targets multiple genes and generates a series of amplicons of approximately 100, 200, 300, 400, and 600 base pairs to ensure that the quality and quantity of input DNA is adequate to yield a valid result. This assay includes negative control DNA and positive control DNAs for both the MBR and mcr. PCR products can be analyzed using standard gel electrophoresis with ethidium bromide staining. A *BCL2* translocation is indicated if any one of the master mixes generates product(s) within the valid size range.

## Background

The *BCL2* t(14;18)(q32;q21) translocation is found in 80-90% of follicular lymphomas and 30% of diffuse large cell lymphomas. The translocation is rarely present in other lymphoproliferative diseases.

The t(14;18) brings about juxtaposition of *BCL2* with the Ig heavy chain joining segment. This leads to a marked increase in expression of *BCL2* driven by the Ig heavy chain gene enhancer. The BCL2 protein inhibits programmed cell death (apoptosis) leading to cell accumulation. The majority of breakpoints on 18q21-22 occur within the major breakpoint region (MBR) in the 3′ untranslated region of exon 3 (60- 70% of the cases), and the minor cluster (mcr) region located 3′ to *BCL2* exon 3 (20-25% of the cases). Some breakpoints occur at distant loci and will not be identified by this particular test. Therefore, a negative result does not completely exclude the presence of a *BCL2/IGH* gene rearrangement in the sample.[1] In comparison, Southern blot analysis requires 1-2 weeks, is significantly less sensitive, and has more restrictive Specimen requirements.

## Specimen Requirements

- 5 mL of peripheral blood, bone marrow biopsy, or bone marrow aspirate anti-coagulated with heparin or EDTA; or,
- Minimum 5 mm cube of tissue; or,
- 2 µg of genomic DNA; or,
- Formalin-fixed, paraffin-embedded (FFPE) tissue or slides.

## Reference

1. PAS Evans et al., *Leukemia* 17: 2298-2301 (2003).
2. JJM van Dongen et al., *Leukemia* 17: 2257-2317 (2003).

EuroClonality **This assay is based on the EuroClonality/BIOMED-2 Concerted Action BMH4-CT98-3936.**


**Figure Legend:** Schematic diagram of the *IGH-BCL2* t(14;18) translocation showing the *BCL2* gene on the left and the Ig heavy chain (*IGH*) gene on the right. Shown are the relative positions and orientations for the major breakpoint region (MBR) primers, the minor cluster region (mcr) primers, and the JH primer, which are included in the 3 *BCL2/*JH master mix tubes.

t(14;18) tube A: 2 *BCL2* MBR primers + 1 JH primer

t(14;18) tube B: 4 *BCL2* 3'MBR primers + 1 JH primer

t(14;18) tube C: 3 *BCL2* mcr primers + 1 JH primer

# Gel and Capillary

*BCL2/*JH Translocation Assay

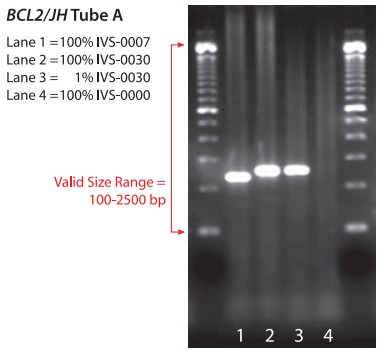
Research Use Only (RUO) Assay

## Reagents

| Controls | Concentration | Units in Assay | Units in Assay MegaKit |
|---|---|---|---|
| IVS-0030 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-P002 Clonal Control DNA | 1600 pg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0031 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0000 Polyclonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| **Master Mixes** | **Target** | **Units in Assay** | **Units in Assay MegaKit** |
| *BCL2/*JH Tube A | *BCL2* MBR + *IGH* JH | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *BCL2/*JH Tube B | *BCL2* 3′ MBR + *IGH* JH | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *BCL2/*JH Tube C | *BCL2* mcr + *IGH* JH | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| Specimen Control Size Ladder | Multiple Genes | 1 x 1500 µL tube | 10 x 1500 µL tubes |

## Gel Electrophoresis Detection

The data shown was generated using the master mix indicated. Amplified products were run on a 2% agarose/TBE gel. Lane 1 is data generated testing an alternative 100% clonal control DNA; lane 2 is data generated testing the recommended 100% clonal control DNA; lane 3 is data generated testing a 1% dilution of the recommended clonal control DNA; and, lane 4 is data generated testing IVS-0000 Polyclonal Control DNA (Cat# 4-092-0010). The IVS-0000 control is used as a negative control for many of our tests. A standard 100 base pair DNA size ladder was run in the lanes flanking the test samples.




## Ordering Information

| Catalog # | Products | Quantity |
|---|---|---|
| 1-309-0020 | *BCL2/*JH Translocation Assay - Gel Detection | 33 reactions |
| 1-309-0040 | *BCL2/*JH Translocation Assay MegaKit - Gel Detection | 330 reactions |

# *BCR/ABL* t(9;22) Translocation Assays

## Assay Use

*BCR/ABL* t(9;22) Translocation Assays are useful for studies involving:

- Identification of chronic myeloid leukemia (CML), acute lymphoblastic leukemia (ALL)
- Monitoring and evaluation of disease recurrence
- Detection and assessment of residual disease
- Evaluation of new research and methods in malignancy studies

## Summary and Explanation of the Test

The master mixes are included in these assay kits used to amplify complementary DNA (cDNA) produced from specimen(s), and positive and negative RNA controls (included). Primers target an internal control transcript (*Abl*) and p190-, p210-, and p230-type transcripts expressed from *BCR-ABL1* translocations. The limit of detection of this assay is approximately one *BCR-ABL1* positive cell in a background of one million normal cells. Amplicon products can be analyzed by capillary electrophoresis or standard gel electrophoresis with ethidium bromide staining. A *BCR-ABL1* translocation is indicated if just one of the 2nd round master mixes (Mix 2b, Mix 2c, Mix 3b, Mix 3c, or Mix 3d) generates product(s) of the valid size. Reagents for RNA extraction and reverse transcription are not included. This assay is compatible with all standard RNA extraction and cDNA synthesis methods. This is a qualitative assay and has not been validated for quantitative use.

## Background

The Philadelphia chromosome (Ph1) is a specific chromosomal abnormality that results from reciprocal t(9;22)(q34;q11) chromosome rearrangements that fuse coding regions of the *BCR* gene, located on chromosome 22, with the *ABL* receptor independent tyrosine kinase gene on chromosome 9.[1,2] *BCR-ABL1* t(9;22) translocations are present in approximately 95% of chronic myeloid leukemia (CML) patients, 20-50% of adult acute lymphoblastic leukemia (ALL) patients, and 2-10% of pediatric ALL patients.[2] Although cytogenetic detection of Ph1 is a hallmark of CML, molecular detection of Ph1-positive cells by nested reverse transcriptase PCR is faster and significantly more sensitive than cytogenetics or other methods. Nearly 50% of cytogenetically Ph1-negative CML cases are positive by reverse transcriptase PCR analysis. This makes reverse transcriptase PCR detection of Ph1-positive cells of value in predicting early disease recurrence and progression for CML and ALL patients that are in apparent clinical remission following bone marrow transplantation.[3] Molecular detection provides the opportunity for early intervention and treatment. Thus, molecular testing for chimeric *BCR-ABL1* transcripts is utilized both in diagnostic evaluation and post-therapeutic monitoring of CMLs and ALLs.

## Specimen Requirements

- 5 mL of peripheral blood, bone marrow biopsy, or bone marrow aspirate anticoagulated with heparin or EDTA; or,
- Archived cells frozen in 10% DMSO + 90% Fetal Bovine Serum (FBS).

## Reference

1. R Kurzrock et al., *Ann. Intern. Med.* 138: 819-30 (2003).
2. JV Melo. *Blood* 88: 2375-2384 (1996).
3. JP Radich et al., *Blood* 85: 2632-2638 (1995).

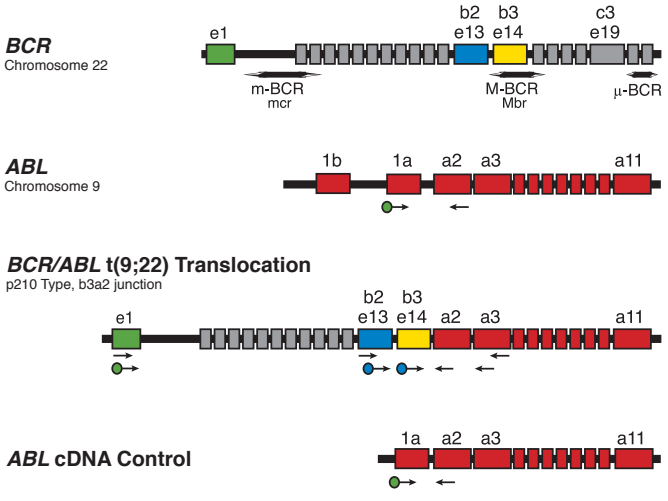



**Figure Legend:** This figure shows the genomic organization of the *BCR* and *ABL* genes on chromosomes 22 and 9, respectively. Boxes represent exon regions of the *ABL* (red boxes) and *BCR* encoding exons (other colors). The solid black line represents intron regions, which have been left incompletely spliced to assist in demarcation of the exon segments. The location of exon regions targeted by labeled and unlabeled primers are indicated by arrows. A p210-type *BCR-ABL1* translocation (b3a2 junction) is depicted in the lower portion of the figure along with the control *ABL* transcript control.

# Gel and Capillary

*BCR/*ABL t(9;22) Translocation Assays

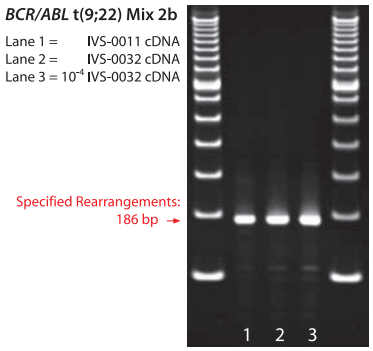
Research Use Only (RUO) Assay

## Reagents

| Controls | Concentration | Units in Assay | Units in Assay MegaKit |
|---|---|---|---|
| IVS-0032 Clonal Control RNA | 400 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0011 Clonal Control RNA | 400 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0035 Clonal Control RNA | 400 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| **Master Mixes** | **Target** | **Units in Assay** | **Units in Assay MegaKit** |
| *BCR/ABL* t(9;22) Mix 1a | *Abl* | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *BCR/ABL* t(9;22) Mix 2a | p190 | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *BCR/ABL* t(9;22) Mix 3a | p210+230 | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *BCR/ABL* t(9;22) Mix 1b | *Abl* | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *BCR/ABL* t(9;22) Mix 2b | p190 | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *BCR/ABL* t(9;22) Mix 2c | p190 | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *BCR/ABL* t(9;22) Mix 3b | p210+230 | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *BCR/ABL* t(9;22) Mix 3c | p210+230 | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *BCR/ABL* t(9;22) Mix 3d | p210+230 | 1 x 1500 µL tube | 10 x 1500 µL tubes |

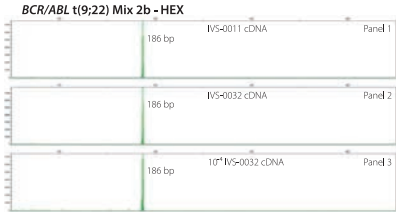
## Gel Electrophoresis Detection

The data shown was generated using the master mix indicated. Amplified products were run on a 6% polyacrylamide/TBE gel. Lane 1 is data generated testing cDNA synthesized from an alternative 100% clonal control RNA; lane 2 is data generated testing cDNA synthesized from the recommended 100% clonal control RNA; lane 3 is data generated testing cDNA synthesized from a $10^{-4}$ dilution of the recommended clonal control RNA. The positive RNA was diluted into the negative control RNA, IVS-0035. A standard 100 base pair DNA size ladder was run in the lanes flanking the test samples.




## Capillary Electrophoresis Detection (ABI)

The data shown was generated on an ABI 3100 instrument using the master mix indicated. Panel 1 represents data generated testing cDNA synthesized from an alternative 100% control RNA; panel 2 represents data generated testing cDNA synthesized from the recommended 100% clonal control RNA; and, panel 3 represents data generated testing cDNA synthesized from a $10^{-4}$ dilution of the recommended clonal control RNA. The positive RNA was diluted into the negative control RNA, IVS-0035 (Cat# 4-089-3070).




## Ordering Information

| Catalog # | Products | Quantity |
|---|---|---|
| 1-310-0010 | *BCR/ABL* t(9;22) Translocation Assay - Gel Detection | 30 reactions |
| 1-310-0020 | *BCR/ABL* t(9;22) Translocation Assay MegaKit - Gel Detection | 300 reactions |
| 1-310-0031 | *BCR/ABL* t(9;22) Translocation Assay - ABI Fluorescence Detection | 30 reactions |
| 1-310-0041 | *BCR/ABL* t(9;22) Translocation Assay MegaKit - ABI Fluorescence Detection | 300 reactions |

# *PML/RARa* t(15;17) Translocation Assays

## Assay Use

*PML/RARα* t(15;17) Translocation Assays are useful for studies involving:

- Identification of acute promyelocytic leukemia (APL)
- Monitoring and evaluation of disease recurrence
- Detection and assessment of residual disease
- Evaluation of new research and methods in malignancy studies

## Summary and Explanation of the Test

Four master mixes are included in these assay kits. Master mixes are used to amplify complementary DNA (cDNA) produced from specimen(s), as well as positive and negative RNA controls (included). Primers target an internal control transcript (*RARA*, formerly known as *RARα*) and the variety of Bcr1, Bcr2, and Bcr3 type transcripts expressed from *PML-RARA* translocations. The limit of detection for this assay is one *PML-RARA* positive cell in a background of one hundred thousand normal cells. Amplicon products can be analyzed by differential fluorescence detection using capillary electrophoresis or standard gel electrophoresis. A *PML-RARA* translocation is indicated if just one of the 2nd round master mixes (Mix 2b or Mix 2c) generates product(s) of the valid size. Reagents for RNA extraction and reverse transcription are not included. This assay is compatible with all standard RNA extraction and cDNA synthesis methods. This is a qualitative assay and has not been validated for quantitative use.

## Background

Acute promyelocytic (M3) leukemia (APL) is a distinct form of acute myeloid leukemia (AML) representing approximately 10% of AMLs. These leukemias often express *PML-RARA* transcripts from t(15;17) chromosomal translocations that fuse the *PML* (or *MYL*) gene on chromosome 15 with the retinoic acid receptor a (*RARA*) gene on chromosome 17.[1,2,3,4] Diagnosis of APL is typically based upon identification of promyelocytes with distinctive morphology plus cytogenetic or molecular detection of these t(15;17) translocations.[4] Three *PML/RARA* translocation patterns have been identified: Type A is the short form (S-form); the breakpoint occurs within the breakpoint cluster region 3 (Bcr3). Type B is the long form (L-form) and the breakpoint occurs within Bcr1. There is a third type B variant or variable form (V-form) whose breakpoint occurs within Bcr2. Identification of the *PML-RARA* translocation is important in APL because it is correlated with responsiveness to treatment with all-trans retinoic acid (ATRA). Patients incorrectly diagnosed with APL by clinical and morphologic criteria alone are typically unresponsive to treatment with ATRA. APL patients are also at risk for disseminated intravascular coagulation (DIC), which can become more severe during conventional chemotherapy. As a result, there is clearly a need for a rapid, sensitive, and reliable molecular assay that identifies *PML-RARA* transcripts associated with APL. This Assay uses both non-nested (master mix 1) and nested (master mixes 2a, 2b, and 2c) reverse transcriptase PCR for faster and significantly more sensitive results than cytogenetics or other methods.

## Specimen Requirements

- 5 mL of peripheral blood, bone marrow biopsy, or bone marrow aspirate anticoagulated with heparin or EDTA; or,
- Archived cells frozen in 10% DMSO + 90% Fetal Bovine Serum (FBS).

## Reference

1. H De Thé et al., *Nature* 347: 558-561 (1990).
2. H De Thé et al., *Cell* 66: 675-684 (1991).
3. A Kakizuka et al., *Cell* 66: 663-674 (1991).
4. WH Miller et al., *Proc. Natl. Acad. Sci.* 89: 2694-2698 (1992).

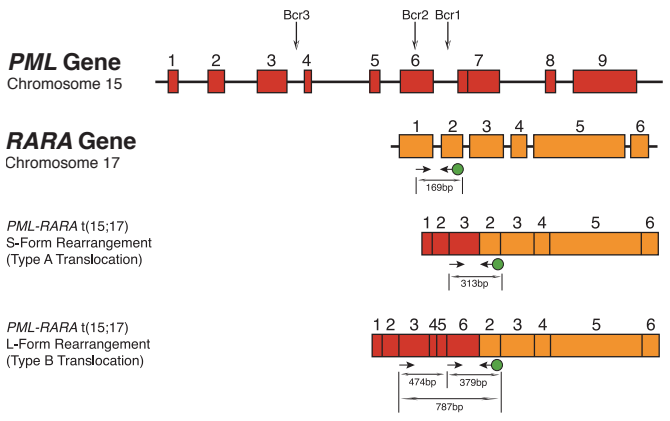



**Figure Legend:** This figure shows the genomic organization of the PML and RARA genes on chromosomes 15 and 17, respectively. Boxes represent exon regions of the *PML* (red boxes) and *RARA* (orange) encoding exons. The solid black line represents intron regions, which were left incompletely spliced to assist in demarcation of the exon segments. Primers are indicated by arrows, and the size of several of the products are indicated below the translocated gene segments. S-form (Bcr3) and L-form (Bcr1) *PML-RARA* translocations are depicted in the lower portion of the figure.

# Gel and Capillary

*PML/RARa* t(9;22) Translocation Assays

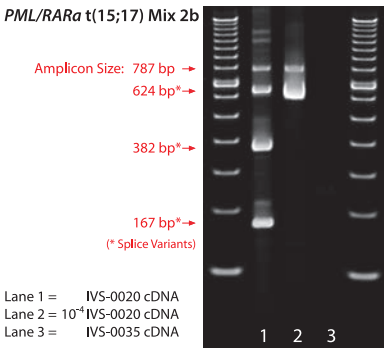
Research Use Only (RUO) Assay

## Reagents

| Controls | Concentration | Units in Assay | Units in Assay MegaKit |
|---|---|---|---|
| IVS-0020 Clonal Control RNA | 400 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0035 Clonal Control RNA | 400 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| **Master Mixes** | **Target** | **Units in Assay** | **Units in Assay MegaKit** |
| *PML/RARα* t(15;17) Mix 1 | *RARA* | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *PML/RARα* t(15;17) Mix 2a | *PML-RARA* | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *PML/RARα* t(15;17) Mix 2b | S- and L-Forms | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *PML/RARα* t(15;17) Mix 2c | L-Form | 1 x 1500 µL tube | 10 x 1500 µL tubes |

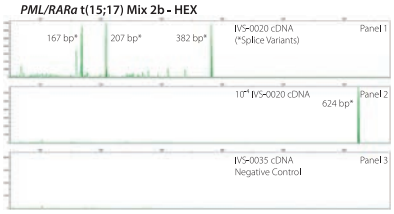
## Gel Electrophoresis Detection

The data shown were generated using the master mix indicated. Amplified products were run on a 6% polyacrylamide/TBE gel. Lane 1 is data generated testing cDNA synthesized from the recommended 100% clonal control RNA; lane 2 is data generated testing cDNA synthesized from a $10^{-4}$ dilution of the recommended clonal control RNA; and, lane 3 is data generated testing cDNA synthesized from the negative control the negative control IVS-0035 Clonal Control RNA (Cat# 4-089-3070). A standard 100 base pair DNA size ladder was run in the lanes flanking the test samples.




## Capillary Electrophoresis Detection (ABI)

The data shown were generated on an ABI 3100 instrument using the master mix indicated. Panel 1 represents data generated testing cDNA synthesized from the recommended 100% clonal control RNA; panel 2 represents data generated testing cDNA synthesized from a $10^{-4}$ dilution of the recommended clonal control RNA; and, panel 3 represents data generated testing cDNA synthesized from the negative control RNA. The positive RNA was diluted into the negative control RNA, IVS-0035 (Cat# 4-089-3070).




## Ordering Information

| Catalog # | Products | Quantity |
|---|---|---|
| 1-311-0010 | *PML/RARα* t(15;17) Translocation Assay - Gel Detection | 30 reactions |
| 1-311-0020 | *PML/RARα* t(15;17) Translocation Assay MegaKit - Gel Detection | 300 reactions |
| 1-311-0011 | *PML/RARα* t(15;17) Translocation Assay - ABI Fluorescence Detection | 30 reactions |
| 1-311-0021 | *PML/RARα* t(15;17) Translocation Assay MegaKit - ABI Fluorescence Detection | 300 reactions |

# *IGH* Somatic Hypermutation Assays v2.0

## Assay Use

*IGH* Somatic Hypermutation Assays are useful for studies involving:

- Identifying clonal rearrangements of the immunoglobulin heavy chain (*IGH*) gene
- Assessing the extent of somatic hypermutation (SHM) in the variable (VH) gene sequence (*IGHV*) in patients with chronic lymphocytic leukemia (CLL) and small lymphocytic lymphoma (SLL)
- Evaluating new research and methods in malignancy studies

## Summary and Explanation of the Test

These assays amplify either genomic DNA or complementary DNA (cDNA) that lies between the upstream leader (VHL) or framework 1 (FR1) regions and the downstream joining (JH) region of the *IGH* gene. The assays employ two different master mixes: Hypermutation Mix 1 and Hypermutation Mix 2. The Hypermutation Mix 1 targets sequences between the leader (VHL) and joining (JH) regions. Therefore the amplicon product(s) span the entire variable (VH) region, which contains all framework (FR) and complementarity-determining regions (CDR). The Hypermutation Mix 2 targets sequences between the framework 1 (FR1) and joining (JH) regions. The resulting amplicons include a portion of the FR1 region to the downstream JH region. The primers that target the VHL and FR1 regions have been redesigned to include a universal sequencing tag at the 5'end. This new design allows for bi-directional sequencing of clonal PCR products with just one sequencing-tag specific forward primer and one JH reverse primer, thus ensuring a more reliable and complete coverage of clonal products. Current ERIC (European Research Initiative on CLL) guidelines recommend bi-directional sequencing when determining the *IGH* SHM status. Positive and negative DNA, positive RNA, as well as an amplification control are included in the assay. Clonality is indicated if any one of the master mixes generates clonal products.

## Background

Immunoglobulin variable heavy chain gene hypermutation status provides important prognostic information for patients with chronic lymphocytic leukemia (CLL) and small lymphocytic lymphoma (SLL). The presence of *IGH* somatic hypermutation (SHM) is defined as greater or equal to 2% difference from the germline variable (VH) gene sequence, whereas less than 2% difference is considered evidence of no somatic hypermutation. This has clinical relevance, as there is a clear distinction in the median survival of patients with and without somatic hypermutation.[2] Hypermutation of the *IGHV* gene is strongly predictive of a good prognosis while lack of mutation predicts a poor prognosis. This assay aids in identification, sequencing, and analysis of somatic hypermutation status of clonal products. PCR products can be analyzed by gel electrophoresis detection or by differential fluorescence detection using capillary electrophoresis followed by gel electrophoresis detection. PCR products are sequenced bidirectionally either directly, after gel extraction, or after cloning into a bacterial vector. The resulting sequence is then compared to *IGH* germline sequences to determine mutational status.

## Specimen Requirements

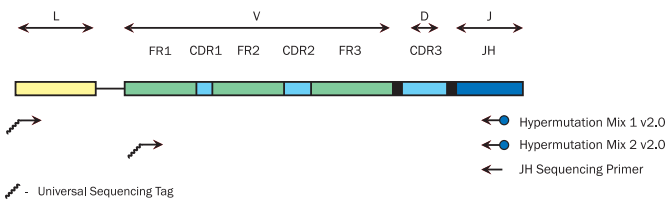
These assays test genomic DNA or cDNA from the following sources:

- 5 mL of peripheral blood, bone marrow biopsy, or bone marrow aspirate anti-coagulated with heparin or EDTA; or,
- Minimum 5 mm cube of tissue; or,
- 2 µg of genomic DNA; or,
- 5 µg of total RNA or mRNA; or,
- 1 µg of cDNA; or,
- Formalin-fixed, paraffin-embedded (FFPE) tissue or slides.

## Reference

1. P Ghia et al., *Leukemia* 21: 1-3 (2007).
2. P Ghia et al., *Blood* 105: 1678-1685 (2005).
3. F Davi et al., *Leukemia* 22: 212-214 (2008).

**Figure Legend:** Simple representation of the organization of a rearranged immunoglobulin heavy chain gene on chromosome 14. Black arrows represent the relative positions of primers that target the conserved Leader (L) and Framework 1 (FR1) regions, and the downstream consensus JH gene segments.



# Gel and Capillary

*IGH* Somatic Hypermutation Assays v2.0

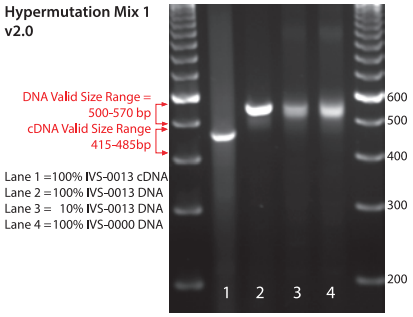
Research Use Only (RUO) Assay

## Reagents

| Controls | Concentration | Units in Assay | Units in Assay MegaKit |
|---|---|---|---|
| IVS-0013 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0013 Clonal Control RNA | 400 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0000 Polyclonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| **Master Mixes** | **Target** | **Units in Assay** | **Units in Assay MegaKit** |
| Hypermutation Mix 1 v2.0 | Leader + JH | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| Hypermutation Mix 2 v2.0 | Framework 1 + JH | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| Specimen Control Size Ladder | Multiple Genes | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| **Primers** | **Target** | **Units in Assay** | **Units in Assay MegaKit** |
| Primer - Hypermutation | Leader + Framework 1 | 1 x 10 µL tube at 100 µM | 5 x 10 µL tube |
| *IGH* JH Primer | JH | 1 x 10 µL tube at 100 µM | 5 x 10 µL tube |

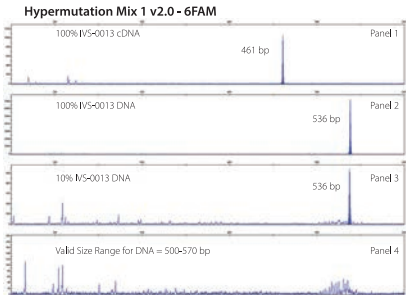
## Gel Electrophoresis Detection

The data shown was generated using the master mix indicated. Amplified products were run on a 2% agarose/TBE gel. Lane 1 is data generated testing cDNA synthesized from the recommended 100% clonal control RNA; lane 2 is data generated testing the recommended 100% clonal control DNA; lane 3 is data generated testing a 10% dilution of the recommended clonal control DNA; and, lane 4 is data generated testing the IVS-0000 Polyclonal Control DNA (Cat# 4-092- 0010). Lanes flanking the samples were loaded with a 100 base pair DNA size standard.




## Capillary Electrophoresis Detection (ABI)

The data shown was generated on an ABI 3130xl instrument using the master mix indicated. Panel 1 displays data generated testing cDNA synthesized from the recommended 100% clonal control RNA; panel 2 displays data generated testing the recommended 100% clonal control DNA; panel 3 displays data generated testing the recommended 10% clonal control DNA; and panel 4 displays data generated testing IVS-0000 Polyclonal Control DNA (Cat# 4-092-0010).




## Ordering Information

| Catalog # | Products | Quantity |
|---|---|---|
| 5-101-0030 | *IGH* Somatic Hypermutation Assay v2.0 - Gel Detection | 33 reactions |
| 5-101-0040 | *IGH* Somatic Hypermutation Assay v2.0 MegaKit - Gel Detection | 330 reactions |
| 5-101-0031 | *IGH* Somatic Hypermutation Assay v2.0 - ABI Fluorescence Detection | 33 reactions |
| 5-101-0041 | *IGH* Somatic Hypermutation Assay v2.0 MegaKit - ABI Fluorescence Detection | 330 reactions |

# *FLT3* Mutation Assays

**These products are not currently available for sale or use in the United States.**

## Assay Use

*FLT3* Mutation Assays are useful for the study of:

- Identifying *FLT3* mutations in patients with AML
- Discriminating between high and low risk patients. *FLT3* mutations portend a worse prognosis for patients with AML. Therefore patients testing positive for *FLT3* mutations may benefit from a more aggressive treatment regimen

## Summary and Explanation of the Test

Acute myeloid leukemia (AML) in general has a poor prognosis. Recent studies have described mutation of the *FLT3* (fms-related tyrosine kinase 3) receptor to be the most important prognostic factor in AML, with *FLT3* mutants having a worse outcome and response to standard chemotherapeutic interventions. Accordingly, identification of an *FLT3* mutation in AML may indicate a need to reassess and modify standard treatment options.

All types of AML can have activating mutations in the *FLT3* gene. Mutation of the *FLT3* receptor, either by internal tandem duplication (ITD) of the juxtamembrane domain or by point mutation of the aspartic acid residue (D835) or isoleucine (I836) in the activation loop of the kinase domain, causes constitutive activation of the *FLT3* receptor.

This test kit includes three master mixes. The ITD and D835 master mixes target the juxtamembrane and kinase domain regions (respectively). The third master mix, the Specimen Control Size Ladder, targets multiple genes and generates a series of amplicons of 100, 200, 300, 400, and 600 base pairs to ensure that the quality and quantity of input DNA is adequate to yield a valid result.

PCR products can be analyzed by capillary electrophoresis with use of ABI instruments or standard agarose TBE gel electrophoresis with ethidium bromide staining.

## Background

Mutations in the fms related tyrosine kinase 3 (*FLT3*) gene are the most common mutations found in acute myeloid leukemia (AML), occurring in approximately 30% of patients at the time of diagnosis, and are characterized by an aggressive phenotype with a high prevalence of relapse.[1,2,3]

The most prevalent and clinically significant type of *FLT3* mutation is an internal tandem duplication (ITD) in the juxtamembrane domain.[4] Many clinical studies have found that *FLT3* ITD mutations are associated with higher concentrations of leukemic cells in both blood and bone marrow, increased incidence of relapse, and decreased overall survival.

The second most common mutation type in the *FLT3* gene is a tyrosine kinase domain (TKD) point mutation in aspartate (D835) or isoleucine (I836). TKD mutations result in constitutive autophosphorylation and activation of *FLT3*.[5,6] TKD mutations have been linked to poor overall survival, but to a lesser extent as compared to ITD mutations.

## Specimen Requirements

- 5 cc of peripheral blood, bone marrow biopsy, or bone marrow aspirate anti-coagulated with heparin or EDTA; or,
- Minimum 5 mm cube of tissue; or,
- 2 µg of genomic DNA; or,
- Formalin-fixed, paraffin-embedded tissue or slides.

## Reference

1. Acute Myeloid Leukemia, Clinical Practice Guidelines in Oncology, National Comprehensive Cancer Network (v.2.2014).
2. Lowenberg, B. et al. "Acute myeloid leukemia." N Engl J Med 341(14):1051-62 (1999).
3. Thiede, C. et al. "Analysis of *FLT3*-activating mutations in 979 patients with acute myelogenous leukemia: association with FAB and identification of subgroups with poor prognosis." Blood 99(12): 4326-35 (2002).
4. Nakao, M. et al. "Internal tandem duplication of the *FLT3* gene found in acute myeloid leukemia." Leukemia 10(12):1911-18 (1996).
5. Yamamoto, Y et al. Activating mutation of D835 within the activation loop of *FLT3* in human hematologic malignancies. Blood, 97(8):2434-9 (2001).
6. Gilliland, DG et al. The roles of *FLT3* in hematopoiesis and leukemia. Blood 100(5):1532-154 (2002).

# Gel and Capillary

*FLT3* Mutation Assays

Research Use Only (RUO) Assay

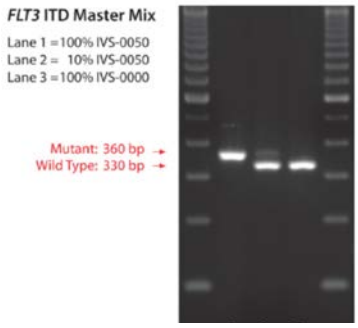
## Reagents

| Controls | Concentration | Units in 33 Reaction Assay | Units in 330 Reaction MegaKit |
|---|---|---|---|
| IVS-0017 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-P001 Clonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| IVS-0000 Polyclonal Control DNA | 200 µg/mL | 1 x 100 µL tube | 5 x 100 µL tubes |
| **Master Mixes** | **Target** | **Units in 33 Reaction Assay** | **Units in 330 Reaction MegaKit** |
| *FLT3* ITD Master Mix | *FLT3* ITD | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| *FLT3* D835 Master Mix | *FLT3* TKD | 1 x 1500 µL tube | 10 x 1500 µL tubes |
| Specimen Control Size Ladder | Multiple Genes | 1 x 1500 µL tube | 10 x 1500 µL tubes |

## Gel Electrophoresis Detection

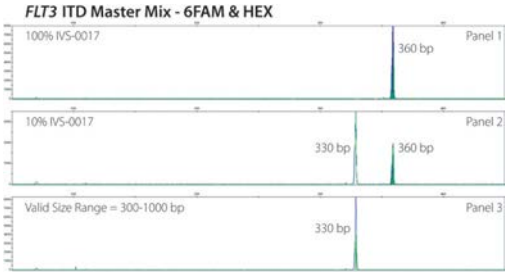
Data was generated using the *FLT3* ITD Master Mix and amplified products were run on a 2% agarose TBE gel. Lane 1 is a *FLT3* ITD control*; lane 2 is a 10% dilution of a *FLT3* ITD control; and lane 3 is IVS-0000, which is representative of a WT product. A standard 100 bp DNA size ladder was run in the lanes flanking the test samples.

*IVS-0050 performs comparable to IVS-0017 clonal control DNA, which is included in the kit as the positive control.




## Capillary Electrophoresis Detection (ABI)

Data was generated using the *FLT3* ITD Master Mix and amplified products were run on an ABI 3100 instrument. Panel 1 is the recommended *FLT3* ITD positive control; panel 2 is data generated testing a 10% dilution of the positive control; and Panel 3 is IVS-0000, which is representative of a WT product.




## Ordering Information

| Catalog # | Products | Quantity |
|---|---|---|
| 1-412-0010 | *FLT3* Mutation Assay - Gel Detection | 33 reactions |
| 1-412-0020 | *FLT3* Mutation Assay MegaKit - Gel Detection | 330 reactions |
| 1-412-0031 | *FLT3* Mutation Assay - ABI Fluorescence Detection | 33 reactions |
| 1-412-0041 | *FLT3* Mutation Assay MegaKit - ABI Fluorescence Detection | 330 reactions |

# Analyte Specific Reagents

WARRANTY AND LIABILITY

Invivoscribe, Inc. (Invivoscribe®) is committed to providing the highest quality products. Invivoscribe® warrants that the products meet or exceed the performance standards described in the Instructions For Use, as to products with such an insert. If a product is covered by product specifications and does not perform as specified, our policy is to replace the product or credit the full purchase price. No other warranties of any kind, expressed or implied, are provided by Invivoscribe®. Invivoscribe® liability shall not exceed the purchase price of the product. Invivoscribe® shall have no liability for direct, indirect, consequential, or incidental damages arising from the use, results of use, or inability to use its products; product efficacy under purchaser controlled conditions in purchaser's laboratory must be established and continually monitored through purchaser defined and controlled processes including but not limited to testing of positive, negative, and blank controls every time a sample is tested. Ordering, acceptance, and use of product constitutes purchaser acceptance of sole responsibility for assuring product efficacy and purchaser agreement to the limitation of liability set forth in this paragraph.

NOTICE: The products in this section are Analyte Specific Reagents; the analytical and performance characteristics are not established. Use of many of these products are covered by one or more of the following: European Patent Number 1549764, European Patent Number 2418287, European Patent Number 2460889, Japanese Patent Number 4708029, United States Patents 6846630, 8178292, 8859748, 10280462, and related pending and future applications. All of these patents and applications are licensed exclusively to Invivoscribe®. Additional patents licensed to Invivoscribe covering some of these products apply elsewhere.

These products require nucleic acid amplification methods such as Polymerase Chain Reaction (PCR). No license under these patents to use amplification processes or enzymes is conveyed expressly or by implication to the purchaser by the purchase of these products.

# Analyte Specific Reagents

Invivoscribe Analyte Specific Reagents (ASRs) target B- and T-cell antigen receptor loci, *FLT3* ITD and TKD, or chromosome translocations (*IGH* - BCL2, BCR-ABL1, PML-RARA).

The ASRs are available as a single tube containing a volume of 1500 **µL**. To ensure the highest quality and reliability of reagents ASRs are manufactured under cGMP and ISO 13485 standards. **The analytical and performance characteristics of the ASRs have not been established.**

Per the current US FDA regulations, ASRs may only be sold to *in vitro* diagnostic manufacturers, CLIA accredited high complexity laboratories, VHA regulated clinical laboratories, and laboratories not intending to use ASRs as a part of a diagnostic test.

**ASRs are not available for sale or use outside of the USA.**

## *FLT3* (FMS-like Tyrosine Kinase 3)

| Description | Catalog # |
|---|---|
| *FLT3* ITD Master Mix - Unlabeled | Please contact Invivoscribe |
| *FLT3* TKD Master Mix - Unlabeled | Please contact Invivoscribe |
| *FLT3* ITD Master Mix - 6FAM & HEX - ASR | A-412-0071 |
| *FLT3* TKD Master Mix - 6FAM - ASR | A-412-0081 |

## *IGH* (Immunoglobulin Heavy Chain Gene Locus)

| Description | Catalog # |
|---|---|
| *IGH* Framework 1 - 6FAM | A-101-0061 |
| *IGH* Framework 2 - Unlabeled | A-101-0070 |
| *IGH* Framework 2 - 6FAM | A-101-0091 |
| *IGH* Framework 3 - Unlabeled | A-101-0080 |
| *IGH* Framework 3 - HEX | A-101-0081 |
| *IGH* FR1 - Unlabeled | A-101-0010 |
| *IGH* FR1 - 6FAM | A-101-0011 |
| *IGH* FR2 - Unlabeled | A-101-0020 |
| *IGH* FR2 - 6FAM | A-101-0101 |
| *IGH* FR3 - Unlabeled | A-101-0030 |
| *IGH* FR3 - HEX | A-101-0031 |
| *IGH* $D_H1$ - 6 - HEX | A-101-0041 |
| *IGH* $D_H7$ - 6FAM | A-101-0051 |

## *IGK* (Immunoglobulin Kappa Light Chain Gene Locus)

| Description | Catalog # |
|---|---|
| *IGK* V - J - Unlabeled | A-102-0010 |
| *IGK* V - J - 6FAM | A-102-0011 |
| *IGK* V - $K_{de}$ - Unlabeled | A-102-0020 |
| *IGK* V - $K_{de}$ - 6FAM | A-102-0021 |

## *IGL* (Immunoglobulin Lambda Light Chain Gene Locus)

| Description | Catalog # |
|---|---|
| *IGL* V - J - 6FAM | A-103-0011 |

## *TRB* (T-Cell Receptor Beta Chain Gene Locus)

| Description | Catalog # |
|---|---|
| *TCRB* V - J1 + 2 - Unlabeled | A-205-0010 |
| *TCRB* V - J1 + 2 - 6FAM & HEX | A-205-0011 |
| *TCRB* V - J2 - Unlabeled | A-205-0020 |
| *TCRB* V - J2 - 6FAM | A-205-0021 |
| *TCRB* D - J1 + 2 - Unlabeled | A-205-0030 |
| *TCRB* D - J1 + 2 - 6FAM & HEX | A-205-0031 |

## *TRD* (T-Cell Receptor Delta Chain Gene Locus)

| Description | Catalog # |
|---|---|
| *TCRD* V - D - J - 6FAM & HEX | A-206-0011 |

## *TRG* (T-Cell Receptor Gamma Chain Gene Locus)

| Description | Catalog # |
|---|---|
| *TCRG* V(2-5,8-11) J 1 + 2+P - 6FAM | A-207-0091 |
| *TCRG* V(1-8,9) J - 6FAM | A-207-0071 |
| *TCRG* V(1-8) J - HEX | A-207-0021 |
| *TCRG* V(1-8,10) J - Unlabeled | A-207-0030 |
| *TCRG* V(1-8,10) J - 6FAM & HEX | A-207-0031 |
| *TCRG* V(9,11) J - Unlabeled | A-207-0040 |
| *TCRG* V(9,11) J - 6FAM & HEX | A-207-0041 |

## *IGH-BCL2* t(14;18)

| Description | Catalog # |
|---|---|
| *BCL2*/$J_H$ Mbr - Unlabeled | A-309-0050 |
| *BCL2*/$J_H$ 3'Mbr - Unlabeled | A-309-0060 |
| *BCL2*/$J_H$ mcr - Unlabeled | A-309-0070 |

## *BCR-ABL1* t(9;22)

| Description | Catalog # |
|---|---|
| *BCR/ABL* e1.1-a3.1 - Unlabeled | A-310-0020 |
| *BCR/ABL* b2.1-a3.1 - Unlabeled | A-310-0030 |
| *BCR/ABL*, e1.2-a2.2 - HEX | A-310-0051 |
| *BCR/ABL*, e1.2-a3.2 - HEX | A-310-0061 |
| *BCR/ABL*, b2.2-a2.2 - 6FAM | A-310-0071 |
| *BCR/ABL*, b2.2-a3.2 - 6FAM | A-310-0081 |
| *BCR/ABL*, b3.2-a3.2 - 6FAM | A-310-0101 |

## *PML-RARA* t(15;17)

| Description | Catalog # |
|---|---|
| *PML/RARa, MYL2-RARa* - Unlabeled | A-311-0020 |
| *PML/RARa, MYL1-RARa* - Unlabeled | A-311-0030 |
| *PML/RARa, PML3-RARa* - Unlabeled | A-311-0040 |

# Controls, Reagents, and Enzymes

# Controls, Reagents, and Enzymes

## Invivoscribe offers an extensive range of General Purpose Reagents (GPRs) and Research Use Only (RUO) nucleic acid controls.

Controls are available in several different formats: plasmid DNA, DNA extracted from tissue or cell lines, or RNA extracted from cell lines. These controls can be purchased in various dilutions or as complete dilution sets and panels for several purposes, such as to help with assay validation, sensitivity or proficiency testing, or troubleshooting.

The following pages will provide an overview of available controls, along with a number of tables and reference guides, to help you decide which Invivoscribe control(s) will be suitable for your application.

# DNA Controls

Every laboratory needs suitable controls (positive and negative) for sensitivity and proficiency testing, as well as for troubleshooting. Since patient samples cannot serve as true controls (due to a lack of characterization and inter-sample variability),

Invivoscribe offers a multitude of high quality, reliable DNA controls manufactured under cGMP conditions.

These controls can be used for most assays targeting B- and T-cell antigen receptor loci, *FLT3* ITD and TKD loci, or *IGH-BCL2, BCR-ABL1,* and *PML-RARa* chromosome translocations.

## Quick Reference for DNA Controls

The vast majority of our high-quality DNA controls, including sensitivity controls and panels, are supplied in aliquots of 100 µL and are adjusted to a final concentration of 200 µg/mL in 1/10 TE (1 mM Tris- HCl (pH 8.0), 0.1 mM EDTA).

| Positive for | Immunoglobulin Rearrangements | | | Mutations | | | Translocations | | T-Cell Receptor Gene Rearrangements | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *IGH* | *IGK* | *IGL* | *IGHV* SHM | *FLT3* ITD | *FLT3* TKD | *IGH-CCND1*** | *IGH-BCL2* | *TRB* | *TRG* | *TRD* |
| IVS-0001 | | | | | | | | ◆ | | | |
| IVS-0004 | | | | | | | | | ◆ | ◆ | |
| IVS-0007 | ◆ | ◆ | ◆ | | | | | ◆ | | | |
| IVS-0008[€] | ❍ | | | | | | | | ◆ | ◆ | |
| IVS-0009 | | | | | | | | | ◆ | ◆ | |
| IVS-0010 | ◆ | ◆ | | | | | ◆ | | | | |
| IVS-0013 | ◆ | ◆ | | | | | | | | | |
| IVS-0019 | ◆ | ◆ | | | | | | | | | |
| IVS-0021 | | ◆ | | | | | | | ◆ | ◆ | ◆ |
| IVS-0024 | ◆ | ◆ | | | | | | | | | |
| IVS-0029 | ◆ | ◆ | | | | | | | | | |
| IVS-0030 [‡] | ◆ | ◆ | | ◆ | | | | | | | |
| IVS-0031 | ◆ | ◆ | | | | | | | | | |
| IVS-P002 | | | | | | | | | | | |
| LymphoQuant B-cell Internal Control | ★ | ★ | | | | | | | | | |
| LymphoQuant T-cell Internal Control | | | | | | | | | ★ | ★ | |
| LymphoTrack B-cell Low Positive Control | ★ | ★ | | | | | | | | | |
| LymphoTrack T-cell Low Positive Control | | | | | | | | | ★ | ★ | |
| *FLT3* ITD Positive Control | | | | | ◆ | | | | | | |
| *FLT3* TKD Positive Control | | | | | | ◆ | | | | | |

◆ Gene rearrangement ❍ Partial *IGH* DH-JH rearrangement ★ Recommended for NGS

[€]This control does not contain a complete *IGH* VH-JH rearrangement and may only be suitable for *IGH* DH-JH rearrangements.

[**]*IGH-CCND1* was previously referred to as *BCL1/*JH

[‡]These controls can be used as SHM positive controls with ≥2% mutational rates compared to the germline sequence.

## Tissue DNA

### Standard Concentrations

The vast majority of our high-quality DNA controls, including sensitivity controls and panels, are supplied in aliquots of 100 µL and are adjusted to a final concentration of 200 µg/mL in 1/10 TE (1 mM Tris- HCl (pH 8.0), 0.1 mM EDTA). This diluent provides sufficient buffering capacity and EDTA to protect the DNA without interfering with the $Mg^{2+}$ concentrations required for robust amplification reactions.

### IVS-0000 Polyclonal Control DNA

Tissue DNA controls are extracted from normal, disease-free tissue and are tested extensively to ensure quality and reproducibility of your test results. IVS-0000 Polyclonal Control DNA consists of genomic DNA isolated from the tissue of normal human tonsils. This control represents an excellent negative control for gene rearrangements, chromosome translocations, and mutation tests and is included in all of our PCR DNA-based assay kits. This DNA is supplied at a volume of 100 µL and at a concentration of 200 µg/mL.

| Catalog # | Description |
|---|---|
| 4-092-0010 | IVS-0000 Polyclonal Control DNA* |

# Controls, Reagents, and Enzymes



## Cell Line DNA

### Reliable Positive Controls

Cell Line DNA controls are extracted from established cell lines grown under cell culture conditions recommended by the supplier. Our controls are tested extensively to ensure quality and reproducibility of your test results. Please note, these controls are for qualitative use only.

**Note:** n/c is used to indicate that the control has not been fully characterized; there may be additional rearrangements, translocations or mutations associated with the control.

### Standard Concentrations

The majority of our high-quality DNA controls, including our sensitivity panels, are supplied in aliquots of 100 µL and are adjusted to a final concentration of 200 µg/mL in 1/10 TE (1 mM Tris-HCl (pH 8.0), 0.1 mM EDTA). This diluent provides sufficient buffering capacity and EDTA to protect the DNA controls without interfering with the $Mg^{2+}$ concentrations required for robust amplification reactions. DNA dilutions are diluted volume to volume (v/v) in our negative control DNA, IVS-0000 Polyclonal Control DNA.

### *FLT3* ITD Positive Control DNA

The *FLT3* ITD Positive Control DNA can be used as a positive control for:

Gene Rearrangements: n/c
Chromosome Translocations: n/c
Mutations: *FLT3* ITD

| Catalog # | Description |
|---|---|
| R0880230 | *FLT3* ITD Positive Control (DNA)* |

### *FLT3* TKD Positive Control DNA

The *FLT3* TKD Positive Control DNA can be used as a positive control for:

Gene Rearrangements: n/c
Chromosome Translocations: n/c
Mutations: *FLT3* TKD

| Catalog # | Description |
|---|---|
| R0880240 | *FLT3* TKD Positive Control (DNA)* |

### *FLT3* Extraction Control

This DNA Control is adjusted to a final concentration of 50 µg/mL in 1/10 TE (1mM Tris-HCL (pH 8.0), 0.1 mM EDTA). This product is a *FLT3* negative cell line DNA that can be used as a DNA extraction control and as a negative control.

| Catalog # | Description |
|---|---|
| R0880250 | *FLT3* Extraction Control* |

### IVS-0001 Clonal Control DNA

IVS-0001 Clonal Control DNA can be used as a positive control for:

Gene Rearrangements: n/c
Chromosome Translocations: *IGH-BCL2* t(14;18) mcr
Mutations: n/c

| Catalog # | Description |
|---|---|
| 4-088-0010 | 100% IVS-0001 Clonal Control DNA* |

### IVS-0004 Clonal Control DNA

IVS-0004 Clonal Control DNA can be used as a positive control for:

Gene Rearrangements: *TRB, TRG*
Chromosome Translocations: n/c
Mutations: n/c

This control is also available as several ready-to-use dilutions into a standard negative control as listed in the table below.

| Catalog # | Description |
|---|---|
| 4-088-0190 | 100% IVS-0004 Clonal Control DNA* |
| 4-088-0210 | 20% IVS-0004 Clonal Control DNA |
| 4-088-0220 | 10% IVS-0004 Clonal Control DNA |
| 4-088-0230 | 5% IVS-0004 Clonal Control DNA |

### IVS-0007 Clonal Control DNA

IVS-0007 Clonal Control DNA can be used as a positive control for:

Gene Rearrangements: *IGH, IGK, IGL*
Chromosome Translocations: *IGH-BCL2* t(14;18) Mbr
Mutations: n/c

This control is also available as several ready-to-use dilutions into a standard negative control as listed in the table below.

| Catalog # | Description |
|---|---|
| 4-088-0370 | 100% IVS-0007 Clonal Control DNA* |
| 4-088-0390 | 20% IVS-0007 Clonal Control DNA |
| 4-088-0400 | 10% IVS-0007 Clonal Control DNA |
| 4-088-0410 | 5% IVS-0007 Clonal Control DNA |
| 4-088-0420 | 1% IVS-0007 Clonal Control DNA |

* These controls are general purpose reagents (GPRs). All others are research use only (RUO).

## Cell Line DNA

### IVS-0008 Clonal Control DNA

IVS-0008 Clonal Control DNA can be used as a positive control for:

Gene Rearrangements: *IGH* DH-JH‡, *TRB, TRG*
Chromosome Translocations: n/c
Mutations: n/c

This control is also available as several ready-to-use dilutions into a standard negative control as listed in the table below.

| Catalog # | Description |
|---|---|
| 4-088-0430 | 100% IVS-0008 Clonal Control DNA* |
| 4-088-0460 | 10% IVS-0008 Clonal Control DNA |
| 4-088-0470 | 5% IVS-0008 Clonal Control DNA |
| 4-088-0480 | 1% IVS-0008 Clonal Control DNA |

### IVS-0009 Clonal Control DNA

IVS-0009 Clonal Control DNA can be used as a positive control for:

Gene Rearrangements: *TRB, TRG*
Chromosome Translocations: n/c
Mutations: n/c

This control is also available as several ready-to-use dilutions into a standard negative control as listed in the table below.

| Catalog # | Description |
|---|---|
| 4-088-0490 | 100% IVS-0009 Clonal Control DNA* |
| 4-088-0500 | 30% IVS-0009 Clonal Control DNA |
| 4-088-0510 | 20% IVS-0009 Clonal Control DNA |
| 4-088-0520 | 10% IVS-0009 Clonal Control DNA |
| 4-088-0530 | 5% IVS-0009 Clonal Control DNA |
| 4-088-0540 | 1% IVS-0009 Clonal Control DNA |

### IVS-0010 Clonal Control DNA

IVS-0010 Clonal Control DNA can be used as a positive control for:

Gene Rearrangements: *IGH, IGK, IGL*
Chromosome Translocations: *IGH-BCL1* t(11;14)
Mutations: n/c

This control is also available as several ready-to-use dilutions into a standard negative control as listed in the table below.

| Catalog # | Description |
|---|---|
| 4-088-0550 | 100% IVS-0010 Clonal Control DNA* |
| 4-088-0560 | 30% IVS-0010 Clonal Control DNA |
| 4-088-0580 | 10% IVS-0010 Clonal Control DNA |
| 4-088-0590 | 5% IVS-0010 Clonal Control DNA |

### IVS-0013 Clonal Control DNA

IVS-0013 Clonal Control DNA can be used as a positive control for:

Gene Rearrangements: *IGH, IGK, IGL*
Chromosome Translocations: n/c
Mutations: n/c

| Catalog # | Description |
|---|---|
| 4-088-0730 | 100% IVS-0013 Clonal Control DNA* |

### IVS-0019 Clonal Control DNA

IVS-0019 Clonal Control DNA can be used as a positive control for:

Gene Rearrangements: *IGH, IGK*
Chromosome Translocations: n/c
Mutations: n/c

This control is also available as several ready-to-use dilutions into a standard negative control as listed in the table below.

| Catalog # | Description |
|---|---|
| 4-088-1090 | 100% IVS-0019 Clonal Control DNA* |
| 4-088-1100 | 30% IVS-0019 Clonal Control DNA |
| 4-088-1110 | 20% IVS-0019 Clonal Control DNA |
| 4-088-1120 | 10% IVS-0019 Clonal Control DNA |
| 4-088-1130 | 5% IVS-0019 Clonal Control DNA |
| 4-088-1140 | 1% IVS-0019 Clonal Control DNA |

### IVS-0021 Clonal Control DNA

IVS-0021 Clonal Control DNA can be used as a positive control for:

Gene Rearrangements: *TRB, TRD, TRG*
Chromosome Translocations: n/c
Mutations: n/c

This control is also available as several ready-to-use dilutions into a standard negative control as listed in the table below.

| Catalog # | Description |
|---|---|
| 4-088-1210 | 100% IVS-0021 Clonal Control DNA* |
| 4-088-1220 | 30% IVS-0021 Clonal Control DNA |
| 4-088-1230 | 20% IVS-0021 Clonal Control DNA |
| 4-088-1240 | 10% IVS-0021 Clonal Control DNA |
| 4-088-1250 | 5% IVS-0021 Clonal Control DNA |
| 4-088-1260 | 1% IVS-0021 Clonal Control DNA |

### IVS-0024 Clonal Control DNA

IVS-0024 Clonal Control DNA can be used as a positive control for:

Gene Rearrangements: *IGH, IGK*
Chromosome Translocations: n/c
Mutations: n/c

This control is also available as a 5% ready-to-use dilution into a standard negative control as listed in the table below.

| Catalog # | Description |
|---|---|
| 4-088-1390 | 100% IVS-0024 Clonal Control DNA* |
| 4-088-1430 | 5% IVS-0024 Clonal Control DNA |

* These controls are general purpose reagents (GPRs). All others are research use only (RUO).

‡ This control does not contain a complete *IGH* VH-JH rearrangement and may only be suitable for *IGH* DH-JH rearrangements.

# Controls, Reagents, and Enzymes



## Cell Line DNA

### IVS-0029 Clonal Control DNA

IVS-0029 Clonal Control DNA can be used as a positive control for:

Gene Rearrangements: *IGH, IGK, IGL*
Chromosome Translocations: n/c
Mutations: n/c

This control is also available as several ready-to-use dilutions into a standard negative control as listed in the table below.

| Catalog # | Description |
|---|---|
| 4-088-1690 | 100% IVS-0029 Clonal Control DNA* |
| 4-088-1700 | 30% IVS-0029 Clonal Control DNA |
| 4-088-1730 | 5% IVS-0029 Clonal Control DNA |

### *IGH* SHM Positive Control DNA

*IGH* SHM Postive Control can be used as a positive control for:

Gene Rearrangements: *IGH*
Chromosome Translocations: n/c
Mutations: *IGH* SHM

| Catalog # | Description |
|---|---|
| 4-088-0008 | *IGH* SHM Positive Control DNA* |

* These controls are general purpose reagents (GPRs). All others are research use only (RUO).

### IVS-0030 Clonal Control DNA

IVS-0030 Clonal Control DNA can be used as a positive control for:

Gene Rearrangements: *IGH, IGK*
Chromosome Translocations: *IGH-BCL2* t(14;18) Mbr
Mutations: n/c

This control is also available as several ready-to-use dilutions into a standard negative control as listed in the table below.

| Catalog # | Description |
|---|---|
| 4-088-1750 | 100% IVS-0030 Clonal Control DNA* |
| 4-088-1760 | 30% IVS-0030 Clonal Control DNA |
| 4-088-1770 | 20% IVS-0030 Clonal Control DNA |
| 4-088-1780 | 10% IVS-0030 Clonal Control DNA |
| 4-088-1790 | 5% IVS-0030 Clonal Control DNA |
| 4-088-1800 | 1% IVS-0030 Clonal Control DNA |

### IVS-0031 Clonal Control DNA

IVS-0031 Clonal Control DNA can be used as a positive control for:

Gene Rearrangements: *IGH, IGK*
Chromosome Translocations: *IGH-BCL2* t(14;18) mcr
Mutations: n/c

This control is also available as several ready-to-use dilutions into a standard negative control as listed in the table below.

| Catalog # | Description |
|---|---|
| 4-088-1810 | 100% IVS-0031 Clonal Control DNA* |
| 4-088-1840 | 10% IVS-0031 Clonal Control DNA |
| 4-088-1860 | 1% IVS-0031 Clonal Control DNA |

## Plasmid DNA

### Reliable Positive Controls

Plasmid DNA controls are engineered to test positive for a specific chromosome translocation or mutation using our assay master mixes. The plasmid is cloned in standard bacterial culture conditions recommended by the supplier. Our controls are tested extensively to ensure quality and reproducibility of your test results. Please note, these controls are for qualitative use only.

**Note:** These controls are assay-specific and may not generate products with other assays.

### Standard Concentrations

Plasmid IVS-P002 is diluted in IVS-0000 Polyclonal Control DNA (at 200 µg/mL in 1/10 TE) to mimic a normal background population.

### IVS-P002 Clonal Control DNA

IVS-P002 Clonal Control DNA can be used as a *IGH-BCL2* t(14;18) (3′ Mbr-JH) positive control for the *BCL2/*JH Tube B master mix included in our *BCL2/*JH Translocation Assay.

Plasmid Concentration: 100 µL @ 1600 pg/mL
Diluent: IVS-0000 Polyclonal Control DNA
Plasmid Size: 4.06 kb

| Catalog # | Description |
|---|---|
| 4-090-0070 | IVS-P002 Clonal Control DNA* |

* These controls are general purpose reagents (GPRs). All others are research use only (RUO).

LymphoTrack®
Low Positive Controls

# Low Positive Controls

Minimal Residual Disease (MRD) testing is a valuable tool that allows investigators to study and monitor multiple myeloma (MM) , chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (AML) and other hematologic diseases. Recent treatment advances have led to significantly increased clinical response and overall survival, but ultimately most subjects will relapse, driving the need for sensitive MRD monitoring. Sensitive and standardized testing such as NGS-based MRD may one day enable identification of those cases that will eventually relapse versus those who are potentially cured. In addition to the need for more sensitive tracking, it is clear that standardized methods are needed. Currently, MRD methods are highly subjective and recommendations are often based on consensus expert-shared knowledge and experience, not on a validated, objective method. Once specific rearrangements have been identified, LymphoTrack assays can be used with LymphoQuant and LymphoTrack Low Positive Controls to track these clonotype populations to a sensitivity as low as $10^{-4}$.

## LymphoTrack® B-cell Low Positive Control

LymphoTrack B-cell Low Positive Control can be used as a control for:

| | |
|---|---|
| Gene Rearrangements: | *IGH, IGK* |
| Chromosome Translocations: | n/c |
| Mutations: | n/c |

| Catalog # | Description |
|---|---|
| 4-088-0098 | LymphoTrack® B-cell Low Positive Control* |

## LymphoTrack® T-cell Low Positive Control

LymphoTrack T-cell Low Positive Control can be used as a control for:

| | |
|---|---|
| Gene Rearrangements: | *TRB, TRG* |
| Chromosome Translocations: | n/c |
| Mutations: | n/c |

| Catalog # | Description |
|---|---|
| 4-088-0108 | LymphoTrack® T-cell Low Positive Control* |

Note: Same product listed on page 54 in MRD Solution section.

*LymphoTrack® Low Positive Controls are research use only (RUO), not for diagnostic procedures.

LymphoQuant®
Internal Controls

# Internal Controls

LymphoQuant T-cell or B-cell Internal Controls may be spiked into specimens to estimate the respective number of clonotype T-cell or B-cell equivalents present. Addition of the LymphoQuant Internal Control to the specimen PCR facilitates clonotype tracking over time without any additional sequencing cost. Consistent use of a LymphoQuant Internal Control enables investigators to objectively monitor the disease over time with a highly standardized, sensitive method. The LymphoTrack MRD software will help researchers that use the LymphoQuant Internal Control, calculate and report an estimated number of clonotype cell equivalents and the percent clonotype in the sample, enabling researchers and pharmaceutical companies to accurately monitor hematologic disease in longitudinal studies.

## LymphoQuant® B-cell Internal Control

LymphoQuant B-cell Internal Control can be used to objectively track Ig clonotypes.

| | |
|---|---|
| Gene Rearrangements: | *IGH, IGK* |
| Chromosome Translocations: | n/c |
| Mutations: | n/c |

| Catalog # | Description |
|---|---|
| 4-088-0118 | LymphoQuant® B-cell Internal Control* |

## LymphoQuant® T-cell Internal Control

LymphoQuant T-cell Internal Control can be used to objectively track TCR clonotypes.

| | |
|---|---|
| Gene Rearrangements: | *TRB, TRG* |
| Chromosome Translocations: | n/c |
| Mutations: | n/c |

| Catalog # | Description |
|---|---|
| 4-088-0128 | LymphoQuant® T-cell Internal Control* |

Note: Same product listed on page 55 in MRD Solution section.

*LymphoQuant® Internal Controls are research use only (RUO), not for diagnostic procedures.

# RNA Controls

## Quick Reference for RNA Controls

### Reliable Assay Controls

Our RNA controls are extracted from well characterized cell lines grown under standard and carefully controlled culture conditions. The controls are tested to ensure linearity and reproducible results. Since this RNA is extracted from cell lines, these controls can be used with any of the standard housekeeping genes.

### Standard Concentrations

Each RNA single control tube (as separate control tube, RNA sensitivity panel and proficiency panel) is supplied in aliquots of 100 µL at a final concentration of 400 µg/mL in water. Each *BCR/ABL* RNA dilution set member is supplied in aliquots of 50 µL at a final concentration of 400 µg/mL in water. To ensure maximum stability, the dilution set should be stored at -85 °C to -65 °C and the number of freeze-thaw cycles should be kept to a minimum.

### RNAs positive for chromosome translocations

| Chromosome Translocation | Clonal Control RNA | Chromosome Translocation | Clonal Control RNA |
|---|---|---|---|
| *BCR-ABL1* t(9;22) p210 e13a2 (b2a2) | IVS-0003 | *CBFB-MYH11* inv(16) | IVS-0015 |
| *BCR-ABL1* t(9;22) p210 e14a2 (b3a2) | IVS-0011 | *E2A-PBX1* t(1;19)(q23;p13) | IVS-0002 |
| *BCR-ABL1* t(9;22) p190 e1a2 | IVS-0032 | *PML-RARA* t(15;17)(q22;q11) | IVS-0020 |

### RNAs negative for chromosome translocations

IVS-0035 can be used as a negative control for our *BCR/ABL* t(9;22) and *PML/RARA* t(15;17) Translocation Assays.

IVS-0035 may be used as a negative control for other chromosome translocation assays, or diluents for other chromosome translocation positive controls. Please do not hesitate to contact us at sales@invivoscribe.com so we can evaluate whether this control may work for your testing needs.

# Controls, Reagents, and Enzymes



## Cell Line RNA

### Reliable Positive and Negative Controls

Cell Line RNA controls are extracted from established cell lines grown under cell culture conditions recommended by the supplier. Our controls are tested extensively to ensure quality and reproducibility of your test results. Please note, these controls are for qualitative use only.

### Standard Concentrations

Our GMP-manufactured high-quality RNA controls, including sensitivity controls and proficiency panel samples, are supplied in aliquots of 100 µL and are adjusted to a final concentration of 400 µg/mL in RNase-free glass-distilled water. The pH of distilled water is slightly acidic; this protects the RNA from hydrolysis. RNA dilutions are diluted volume to volume in our negative control RNA, IVS-0035 Clonal Control RNA.

### IVS-0002 Clonal Control RNA

IVS-0002 Clonal Control RNA can be used as a positive control for the chromosome translocation: *E2A-PBX1* t(1;19) (q23;p13).

| Catalog # | Description |
|---|---|
| 4-089-0100 | IVS-0002 Clonal Control RNA* |

### IVS-0003 Clonal Control RNA

IVS-0003 Clonal Control RNA can be used as a positive control for the chromosome translocation: *BCR-ABL1* t(9;22) p210 e13a2 (b2a2).

This control is also available as several ready-to-use dilutions into a standard negative control as listed in the table below.

| Catalog # | Description |
|---|---|
| 4-089-0190 | IVS-0003 Clonal Control RNA* |
| 4-089-0200 | $10^{-1}$ IVS-0003 Clonal Control RNA |
| 4-089-0210 | $10^{-2}$ IVS-0003 Clonal Control RNA |
| 4-089-0220 | $10^{-3}$ IVS-0003 Clonal Control RNA |
| 4-089-0230 | $10^{-4}$ IVS-0003 Clonal Control RNA |
| 4-089-0240 | $10^{-5}$ IVS-0003 Clonal Control RNA |
| 4-089-0250 | $10^{-6}$ IVS-0003 Clonal Control RNA |

### IVS-0011 Clonal Control RNA

IVS-0011 Clonal Control RNA can be used as a positive control for the chromosome translocation: *BCR-ABL1* t(9;22) p210 e14a2 (b3a2).

This control is also available as several ready-to-use dilutions into a standard negative control as listed in the table below.

| Catalog # | Description |
|---|---|
| 4-089-0910 | IVS-0011 Clonal Control RNA* |
| 4-089-0920 | $10^{-1}$ IVS-0011 Clonal Control RNA |
| 4-089-0930 | $10^{-2}$ IVS-0011 Clonal Control RNA |
| 4-089-0940 | $10^{-3}$ IVS-0011 Clonal Control RNA |
| 4-089-0950 | $10^{-4}$ IVS-0011 Clonal Control RNA |
| 4-089-0960 | $10^{-5}$ IVS-0011 Clonal Control RNA |

### IVS-0015 Clonal Control RNA

IVS-0015 Clonal Control RNA can be used as a positive control for the chromosome translocation: *CBFB-MYH11* inv(16)

| Catalog # | Description |
|---|---|
| 4-089-1270 | IVS-0015 Clonal Control RNA* |

### IVS-0020 Clonal Control RNA

IVS-0020 Clonal Control RNA can be used as a positive control for the chromosome translocation: *PML-RARA* t(15;17) L-Form.

This control is also available as several ready-to-use dilutions into a standard negative control as listed in the table below.

| Catalog # | Description |
|---|---|
| 4-089-1720 | IVS-0020 Clonal Control RNA* |
| 4-089-1730 | $10^{-1}$ IVS-0020 Clonal Control RNA |
| 4-089-1740 | $10^{-2}$ IVS-0020 Clonal Control RNA |
| 4-089-1750 | $10^{-3}$ IVS-0020 Clonal Control RNA |
| 4-089-1760 | $10^{-4}$ IVS-0020 Clonal Control RNA |

### IVS-0032 Clonal Control RNA

IVS-0032 Clonal Control RNA can be used as a positive control for the chromosome translocation: *BCR-ABL1* t(9;22) p190 e1a2.

This control is also available as several ready-to-use dilutions into a standard negative control as listed in the table below.

| Catalog # | Description |
|---|---|
| 4-089-2800 | IVS-0032 Clonal Control RNA* |
| 4-089-2810 | $10^{-1}$ IVS-0032 Clonal Control RNA |
| 4-089-2820 | $10^{-2}$ IVS-0032 Clonal Control RNA |
| 4-089-2830 | $10^{-3}$ IVS-0032 Clonal Control RNA |
| 4-089-2840 | $10^{-4}$ IVS-0032 Clonal Control RNA |
| 4-089-2850 | $10^{-5}$ IVS-0032 Clonal Control RNA |
| 4-089-2860 | $10^{-6}$ IVS-0032 Clonal Control RNA |

### IVS-0035 Clonal Control RNA

IVS-0035 Clonal Control RNA can be used as a negative control for our *BCR/ABL1* t(9;22) and *PML/RARa* t(15;17) Translocation Assays.

| Catalog # | Description |
|---|---|
| 4-089-3070 | IVS-0035 Clonal Control RNA* |

* These controls are general purpose reagents (GPRs). All others are research use only (RUO).

## *BCR/ABL* RNA Dilution Sets

Our *BCR/ABL* b2a2, b3a2, and e1a2 RNA Dilution Sets consist of RNA that has been extracted from *BCR-ABL1* expressing and *BCR-ABL1* negative cell lines. Each set is composed of several dilutions ($10^{-1}$, $10^{-2}$, $10^{-3}$, $10^{-4}$, $10^{-5}$) of the *BCR-ABL1* positive RNA diluted (v/v) into RNA purified from a cell line that does not contain a *BCR-ABL1* translocation. Also included in these sets is a 100% *BCR-ABL1* negative RNA.

The individual *BCR/ABL* b2a2, b3a2, and e1a2 RNA Dilution Sets can be used as reference and validation materials with assays that target the main transcripts of *BCR-ABL1* t(9;22) translocations: p210 (e13a2 (b2a2), e14a2 (b3a2), and p190 (e1a2). These products may be used as the following:

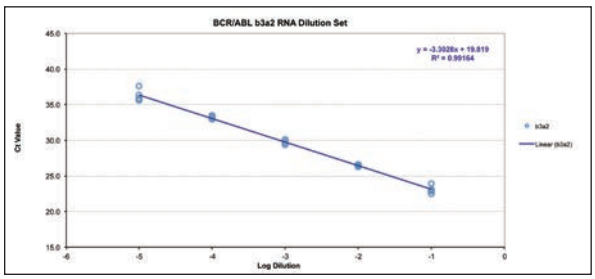
- Routine testing controls for cDNA synthesis, amplification and detection
- Controls to establish a standard reference curve
- Proficiency controls
- Sensitivity controls for specific target assays

### Data

Plot of Ct values (5 replicates) for the $10^{-1}$, $10^{-2}$, $10^{-3}$, $10^{-4}$, and $10^{-5}$ dilutions.

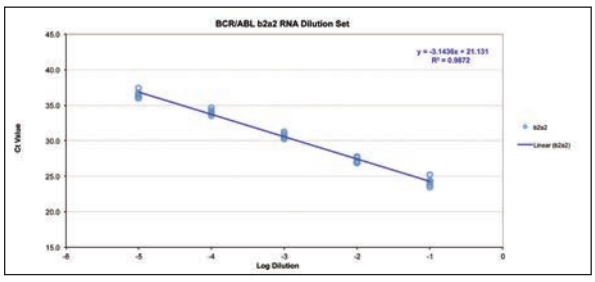
**Ordering Information – e14a2 (b3a2)**

| Catalog # | Description |
|---|---|
| 4-085-0210 | *BCR/ABL* b3a2 RNA Dilution Set ($10^{-1}$, $10^{-2}$, $10^{-3}$, $10^{-4}$, $10^{-5}$ dilutions and negative) |



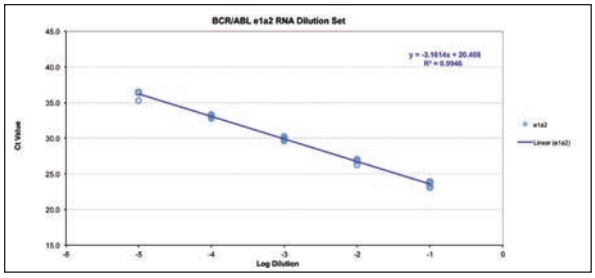

**Ordering Information – e13a2 (b2a2)**

| Catalog # | Description |
|---|---|
| 4-085-0310 | *BCR/ABL* b2a2 RNA Dilution Set ($10^{-1}$, $10^{-2}$, $10^{-3}$, $10^{-4}$, $10^{-5}$ dilutions and negative) |




**Ordering Information – e1a2**

| Catalog # | Description |
|---|---|
| 4-085-0110 | *BCR/ABL* e1a2 RNA Dilution Set ($10^{-1}$, $10^{-2}$, $10^{-3}$, $10^{-4}$, $10^{-5}$ dilutions and negative) |




These controls are research use only (RUO), not for diagnostic procedures.

# Control Panels

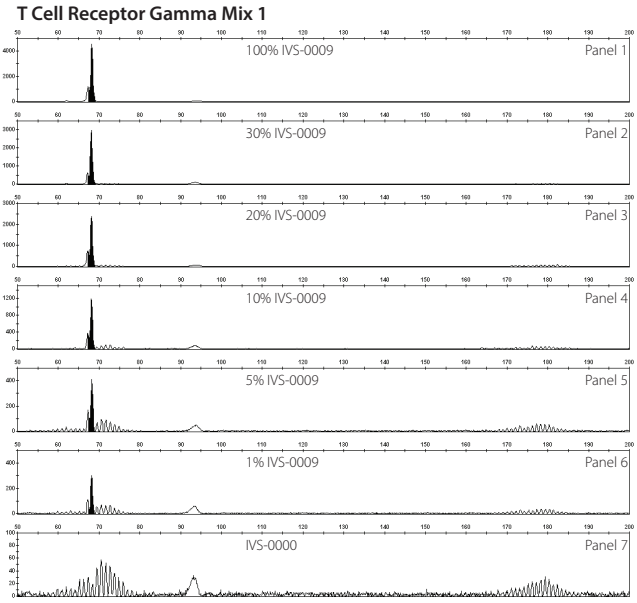
## DNA and RNA Sensitivity Panels

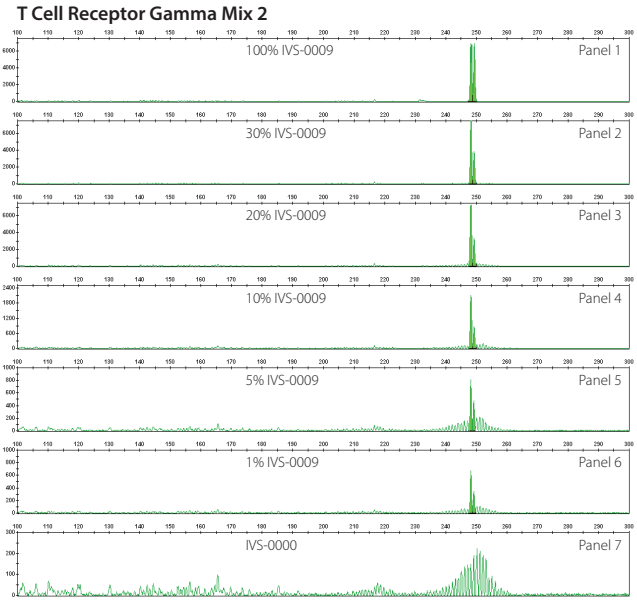
### DNA Sensitivity Panels

DNA sensitivity panels are 6 member panels that consist of 100% clonal DNA extracted from a positive control cell line and 30%, 20%, 10%, 5%, and 1% dilutions of the positive clonal DNA diluted (v/v) into our standard negative control DNA, IVS-0000 Polyclonal Control DNA. Each tube contains 100 µL of DNA at a concentration of 200 µg/mL in 1/10 TE buffer (1 mM Tris-HCl pH 8.0, 0.1 mM EDTA). This diluent provides sufficient buffering capacity and EDTA to protect the DNA controls without interfering with the $Mg^{2+}$ concentrations required for robust amplification reactions. Please note, these controls are for qualitative use only.

### RNA Sensitivity Panels

RNA sensitivity panels are 7 member panels that consist of 100% clonal RNA extracted from a positive control cell line and $10^{-1}$, $10^{-2}$, $10^{-3}$, $10^{-4}$, $10^{-5}$, and $10^{-6}$ (1:10 – 1:1 000 000) dilutions of the positive clonal RNA diluted (v/v) into our standard negative control RNA, IVS-0035 Clonal Control RNA. Each tube contains 100 µL of RNA at 400 µg/mL in RNase-free glass-distilled water. The pH of distilled water is slightly acidic thereby protecting the RNA from hydrolysis. Please note, these controls are for qualitative use only.




This data was generated testing a Sensitivity Panel for IVS-0009 Clonal Control DNA using the master mixes listed. PCR products were run on an ABI 3130xL capillary electrophoresis instrument for differential fluorescence detection and data analyses. Panel 7 shows the polyclonal Gaussian distributions expected from our negative control IVS-0000 Polyclonal Control DNA. Data in the other panels are tests of positive control samples at the dilutions indicated. Clonal peaks (highlighted) are clearly evident in all of the positive sample panels.

# Controls, Reagents, and Enzymes



## DNA Sensitivity Panels

| Catalog # | Description | Can be used as a positive control for: |
|---|---|---|
| 4-086-0040 | Sensitivity Panel for IVS-0004 Clonal Control DNA | *TRB, TRG* |
| 4-086-0070 | Sensitivity Panel for IVS-0007 Clonal Control DNA | *IGH, IGK, IGL, IGH-BCL2* t(14;18) Mbr |
| 4-086-0090 | Sensitivity Panel for IVS-0009 Clonal Control DNA | *TRB, TRG* |
| 4-086-0100 | Sensitivity Panel for IVS-0010 Clonal Control DNA | *IGH, IGK, IGL, IGH-CCND1* t(11;14) |
| 4-086-0190 | Sensitivity Panel for IVS-0019 Clonal Control DNA | *IGH, IGK* |
| 4-086-0210 | Sensitivity Panel for IVS-0021 Clonal Control DNA | *TRB, TRD, TRG* |
| 4-086-0300 | Sensitivity Panel for IVS-0030 Clonal Control DNA | *IGH, IGK, IGH-BCL2* t(14;18) Mbr |

## RNA Sensitivity Panels

| Catalog # | Description | Can be used as a positive control for: |
|---|---|---|
| 4-087-0030 | Sensitivity Panel for IVS-0003 Clonal Control RNA | *BCR-ABL1* t(9;22) p210 e13a2 (b2a2) |
| 4-087-0110 | Sensitivity Panel for IVS-0011 Clonal Control RNA | *BCR-ABL1* t(9;22) p210 e14a2 (b3a2) |
| 4-087-0150 | Sensitivity Panel for IVS-0015 Clonal Control RNA | *CBFB/MYH11* inv16 |
| 4-087-0200 | Sensitivity Panel for IVS-0020 Clonal Control RNA | *PML-RARA* t(15;17) L-form |
| 4-087-0320 | Sensitivity Panel for IVS-0032 Clonal Control RNA | *BCR-ABL1* t(9;22) p190 e1a2 |

## Proficiency Panel for *BCR-ABL1* t(9;22)

This 10 member panel consists of 100% clonal control RNA extracted from three *BCR-ABL1* positive cell lines as well as $10^{-2}$ (1:100) and $10^{-4}$ (1:10,000) dilutions (v/v) of these positive RNAs diluted into a normal (*BCR-ABL1* negative) control RNA, IVS-0035 Clonal Control RNA. A sample of 100% IVS-0035 Clonal Control RNA is also included. All three cell lines (IVS-0003, IVS-0011, and IVS-0032) carry a t(9;22) translocation. One of the cell lines, IVS-0032, encodes for p190-type (ALL-associated) transcript with e1a2 junctions. The other two cell lines both encode for p210-type (CML-associated) transcripts. One of the p210-type translocations, IVS-0003, harbors a e13a2 (b2a2) junction and the other, IVS-0011, harbors a e14a2 (b3a2) junction. This proficiency panel is used to validate tests that identify *BCR-ABL1* t(9;22) translocations and is designed to be used with the *BCR/ABL* t(9;22) Translocation Assay Kits.

### RNA Proficiency Panel

| Qty | Description | Chromosome Translocation |
|---|---|---|
| 1 | IVS-0003 Clonal Control RNA<br>$10^{-2}$ IVS-0003 Clonal Control RNA<br>$10^{-4}$ IVS-0003 Clonal Control RNA | *BCR-ABL1*<br>p210 e13a2 (b2a2) |
| 1 | IVS-0011 Clonal Control RNA<br>$10^{-2}$ IVS-0011 Clonal Control RNA<br>$10^{-4}$ IVS-0011 Clonal Control RNA | *BCR-ABL1*<br>p210 e14a2 (b3a2) |
| 1 | IVS-0032 Clonal Control RNA<br>$10^{-2}$ IVS-0032 Clonal Control RNA<br>$10^{-4}$ IVS-0032 Clonal Control RNA | *BCR-ABL1*<br>p190 e1a2 |
| 1 | IVS-0035 Clonal Control RNA | *BCR-ABL1*<br>Negative |

### Ordering Information

| Catalog # | Description |
|---|---|
| 4-310-0100 | Proficiency Panel for the *BCR/ABL* t(9;22) Translocation Assay |

These products are research use only (RUO), not for diagnostic procedures.

# Master Mix Controls

These master mixes serve as control for many of our DNA assays to ensure that sample DNA is of sufficient quality and integrity to generate a valid result.

## Amplification Control Master Mix

Our Amplification Control master mix targets the *HLA*-DQa locus and generates a product of 235 basepairs in size from human genomic DNA. This control is available in unlabeled (for Gel Detection) and fluorescence labeled format (for ABI Fluorescence Detection, 6FAM).

| Catalog # | Description |
|---|---|
| 2-096-0010 | Amplification Control Master Mix - Unlabeled* |
| 2-096-0011 | Amplification Control Master Mix - 6FAM* |

## Specimen Control Size Ladder

Our Specimen Control Size Ladder master mix targets four different housekeeping genes producing products of approximately 100, 200, 300, 400, and 600 basepairs in size to ensure that the quality and quantity of the sample DNA is adequate to yield a valid result with the specific assay(s).

This master mix is based on the BIOMED-2 Concerted Action BMH4-CT98-3936 from the EuroClonality Group and is available for Gel Detection (unlabeled) or ABI detection (labeled with 6FAM).

| Catalog # | Description |
|---|---|
| 2-096-0020 | Specimen Control Size Ladder - Unlabeled* |
| 2-096-0021 | Specimen Control Size Ladder - 6FAM* |

*These master mixes are general purpose reagents (GPRs).

# Reagents

## ABI Detection Reagents

### Reagents for ABI Fluorescence Detection

Invivoscribe also offers highly deionized (Hi-Di) Formamide with ROX size standards for ABI fluorescence detection with the ABI 310 or 3100 series. Hi-Di Formamide is used to stabilize single strands of denatured PCR amplicons. The ROX size standards are fluorescent labeled DNA standards which cover the 50 to 400 basepair size range. Sizes of the individual standards are: 50, 60, 90, 100, 120, 150, 160, 180, 190, 200, 220, 240, 260, 280, 290, 300, 320, 340, 360, 380, and 400 basepairs.

For samples tested on an ABI 310 or 3100 series, we recommend using 10 µL of the Hi-Deionized Formamide with ROX Size Standards mixture for each microliter of PCR product. Please note that the ABI 310 and 3100 series require different concentrations of ROX size standards and the different Hi-Deionized Formamide with ROX Size Standards cannot be used interchangeably.

For samples tested on an ABI 3500 series, GeneScan™ 600® LIZ dye Size Standard v2.0 can be purchased from Thermo Fisher Scientific.

**Ordering Information**

| Catalog # | Description |
|---|---|
| 6-098-0051 | Hi-Deionized Formamide with ROX Size Standard (ABI 310), 1 mL |
| 6-098-0061 | Hi-Deionized Formamide with ROX Size Standard (ABI 3100), 1 mL |
| Available through Thermo Fisher Scientific®: 4408399 | GeneScan™ 600 LIZ® dye v2.0 Standard (ABI 3500), 800 reactions |

For research use only (RUO), not for diagnostic procedures.

# Enzymes

## EagleTaq DNA Polymerase

**Note: This product is for sale and use in the European Economic Area only. It is not to be resold or transferred to another party. See also Notice on page 132.**

### EagleTaq DNA Polymerase

EagleTaq DNA Polymerase can be used to obtain highly specific and sensitive PCR amplification products. This enzyme has been proven to minimize extension of non-specifically bound primers. Obtain reliable results by using the gold standard of hot start polymerases for robust performance.

**Ordering Information**

| Catalog # | Description |
|---|---|
| 6-097-0100 | EagleTaq DNA Polymerase 1000 U, 5 U/µL |

For research use only (RUO), not for diagnostic procedures.

# Custom Products

WARRANTY AND LIABILITY

Invivoscribe, Inc. (Invivoscribe®) is committed to providing the highest quality products. Invivoscribe® warrants that the products meet or exceed the performance standards described in the Instructions For Use, as to products with such an insert. If a product is covered by product specifications and does not perform as specified, our policy is to replace the product or credit the full purchase price. No other warranties of any kind, expressed or implied, are provided by Invivoscribe®. Invivoscribe® liability shall not exceed the purchase price of the product. Invivoscribe shall have no liability for direct, indirect, consequential, or incidental damages arising from the use, results of use, or inability to use its products; product efficacy under purchaser controlled conditions in purchaser's laboratory must be established and continually monitored through purchaser defined and controlled processes including but not limited to testing of positive, negative, and blank controls every time a sample is tested. Ordering, acceptance, and use of product constitutes purchaser acceptance of sole responsibility for assuring product efficacy and purchaser agreement to the limitation of liability set forth in this paragraph.

# Custom Products

## Customized Products to Meet Your Needs

The Invivoscribe team of experts can help develop your ideas into customized products. Allow us to partner with you to take a basic concept through design, development, validation, regulatory approval (if applicable), and release. For more information, please call our San Diego office at +1 858.224.6600 or send an e-mail to sales@invivoscribe.com.

## Custom Designed Assays

In response to the FDA announcing its intention to dramatically expand its regulatory oversight of laboratory developed tests (LDTs), Invivoscribe is partnering with laboratories worldwide to help facilitate the conversion of LDTs into FDA-cleared assays, as we know the barriers to bringing new assays online are often the availability of resources and the cost of validation.

By leveraging the power of our regulatory expertise, provided through each milestone, we can help ensure safety, efficacy and quality. Our customizable reagent manufacturing capabilities can reduce your LDT costs and lead to higher-quality testing.

To date, we have partnered with more than 40 laboratories around the world to develop, validate, and launch a variety of molecular products. A number of these partnerships have also led to the release of US and CE-marked *in vitro* diagnostic products and services. Learn how Invivoscribe can help you develop assays for new products, services, and novel applications.

## Custom Controls and Validation Panels

We offer a large selection of well-characterized DNA and RNA controls that are used to define the performance characteristics of a wide variety of molecular reagents. To address your specific requirements, we can partner with you to design, validate, and provide custom controls and validation panels. If necessary, we are willing to acquire, characterize, and engineer custom controls for your specific application. We can produce DNA, RNA, or cDNA at any specified concentration, dilution, or volume. Please contact us with your requirements and we will be happy to provide controls to suit your needs.

## Invivoscribe is a Comprehensive Partner for Companion Diagnostic Development

From biomarker identification through commercialization, Invivoscribe has expertise at every stage of companion diagnostics development.

- **Discovery & Patient Stratification:** We offer comprehensive gene panels to identify biomarkers and define patient populations, thus reducing development costs and improving the success of clinical trials.
- **Clinical Trials:** Our network of global laboratories accelerates sample acquisition and harmonizes testing to ensure accurate results.
- **Regulatory Approval:** Our in-house experts have experience seeking approval with global agencies.
- **Commercialization:** Our cGMP manufacturing expertise and distribution channels allow approved CDx to reach all global markets.

Invivoscribe is an ISO 13485-accredited and FDA/CDRH registered medical device manufacturer with a long record of successful partnerships. We are the industry-leading assay and software development company, providing full QSR design control and a complete range of cGMP manufactured assays, controls, reagents, and services to CLIA-accredited clinical laboratory and pharmaceutical communities.

Please contact us at +1 858.224.6600 or sales@invivoscribe.com for more details about partnering with Invivoscribe for the development and manufacturing of companion diagnostics, *in vitro* diagnostics, molecular reagents, and/or nucleic acid controls.

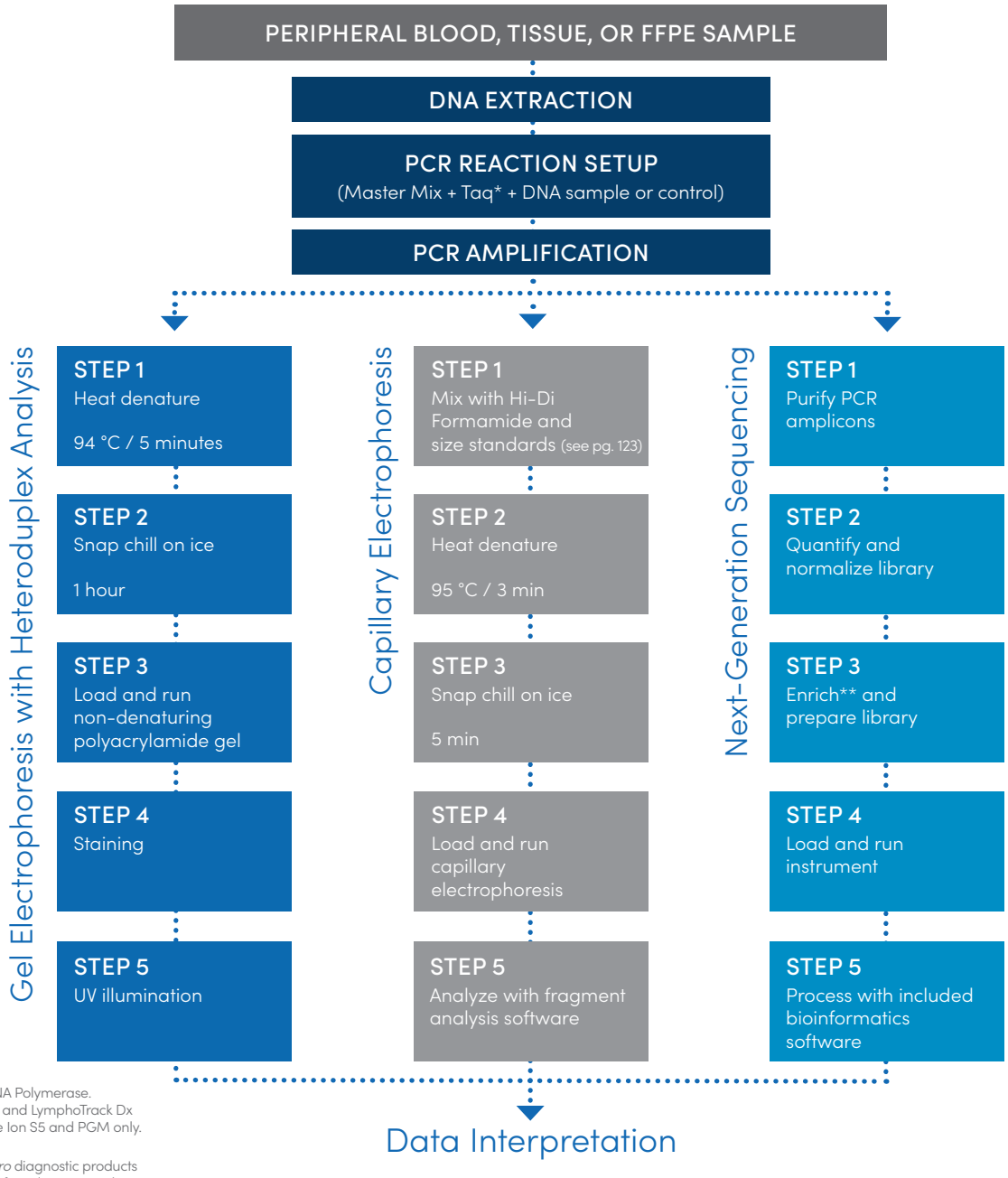
WARRANTY AND LIABILITY

Invivoscribe, Inc. (Invivoscribe®) is committed to providing the highest quality products. Invivoscribe® warrants that the products meet or exceed the performance standards described in the Instructions For Use, as to products with such an insert. If a product is covered by product specifications and does not perform as specified, our policy is to replace the product or credit the full purchase price. No other warranties of any kind, expressed or implied, are provided by Invivoscribe®. Invivoscribe® liability shall not exceed the purchase price of the product. Invivoscribe® shall have no liability for direct, indirect, consequential, or incidental damages arising from the use, results of use, or inability to use its products; product efficacy under purchaser controlled conditions in purchaser's laboratory must be established and continually monitored through purchaser defined and controlled processes including but not limited to testing of positive, negative, and blank controls every time a sample is tested. Ordering, acceptance, and use of product constitutes purchaser acceptance of sole responsibility for assuring product efficacy and purchaser agreement to the limitation of liability set forth in this paragraph.

# Reference

The Invivoscribe European Conformity marked *in vitro* diagnostics (CE-IVD) and Research Use Only (RUO) clonality assays detect clonal populations in just a few easy steps. These steps include PCR amplification of the immunoglobulin or T-cell receptor genes of interest, followed by detection with non-denaturing polyacrylamide gels, capillary electrophoresis, or next-generation sequencing using an Illumina® MiSeq®, Thermo Fisher Scientific® Ion S5™ or PGM™ instrument. A flowchart illustrating this workflow is shown below.

## Clonality Testing Workflow




*Or equivalent DNA Polymerase.
** For LymphoTrack and LymphoTrack Dx Assays run on the Ion S5 and PGM only.

CE-marked *in vitro* diagnostic products are not available for sale or use within North America.

# LymphoTrack Dx and LymphoTrack Workflow Summary

## Illumina® MiSeq®

**STEP 1**
Prepare DNA samples

**STEP 2**
Run PCR with a LymphoTrack Assay

**STEP 3**
Purify amplicons

**STEP 4**
Quantify and prepare library

**STEP 5**
Load onto MiSeq® flow cell and run

**STEP 6**
Process raw data with our included bioinformatics software

**STEP 7**
Analyze results

NOTICE: The LymphoTrack Dx Assays are *in vitro* diagnostic products and are not available for sale or use within North America.

*Image courtesy of Illumina, Inc.

LymphoTrack Dx and LymphoTrack Workflow Summary

# Thermo Fisher Scientific® Ion S5/PGM™

## STEP 1
Prepare DNA samples

## STEP 2
Run PCR with a LymphoTrack Assay

## STEP 3
Purify amplicons

## STEP 4
Quantify, prepare, and enrich library

## STEP 5
Load onto Ion S5 /PGM™ chip and run

## STEP 6
Process raw data with our included bioinformatics software

## STEP 7
Analyze results

NOTICE: The LymphoTrack Dx Assays are *in vitro* diagnostic products and are not available for sale or use within North America.

*Image courtesy of Thermo Fisher Scientific

# Next-Generation Sequencing Menu

Invivoscribe offers LymphoTrack and LymphoTrack Dx Assays for the analysis of B- and T-cell clonality, somatic hypermutation, and minimal residual disease studies**. Assays are designed for use on both industry standard next-generation sequencing (NGS) platforms: the Illumina® MiSeq® and Thermo Fisher Scientific® Ion PGM™ and Ion S5™ instruments.

Invivoscribe assays for the Illumina® MiSeq® platform offer the ability to analyze up to twenty two samples and two controls per gene target and the multiplexing capabilities to generate a sequencing library that combines amplicons from different Invivoscribe LymphoTrack and LymphoTrack Dx Assays onto the same flow cell. Our included software then sorts and assigns the correct sequences to their corresponding sample.

Invivoscribe assays for the Ion PGM™ and Ion S5™ platform offer the ability to analyze up to ten samples and two controls per gene target and the multiplexing capability to generate a sequencing library that combines amplicons from different Invivoscribe LymphoTrack and LymphoTrack Dx Assays onto the same sequencing chip, reducing per sample testing costs.

All LymphoTrack and LymphoTrack Dx Assays allow for fast and easy analysis and data visualization using the included bioinformatics software. The LymphoTrack software sorts and assigns the sequences to their corresponding sample and provides information such as the prevalence, gene segment usage, and the mutation rate (*IGH* Leader and *IGH* FR1 only). In addition, the Invivoscribe Minimal Residual Disease (MRD) Software allows for clonotype sequences to be tracked in subsequent samples for research applications.

The table below indicates which LymphoTrack (Research Use Only) and LymphoTrack Dx (CE-IVD Marked) Assays are currently available in 2020.

| CE-Marked IVD Assays | MiSeq® | Ion S5/PGM™ |
|---|---|---|
| LymphoTrack® Dx *IGHV* Leader Somatic Hypermutation Assays | AVAILABLE | NOT AVAILABLE |
| LymphoTrack® Dx *IGH* FR1 Assays | AVAILABLE | AVAILABLE |
| LymphoTrack® Dx *IGH* FR2 Assays | AVAILABLE | AVAILABLE |
| LymphoTrack® Dx *IGH* FR3 Assays | AVAILABLE | AVAILABLE |
| LymphoTrack® Dx *IGH* FR1/2/3 Assays | AVAILABLE | AVAILABLE |
| LymphoTrack® Dx *IGK* Assays | AVAILABLE | AVAILABLE |
| LymphoTrack® Dx *TRG* Assays | AVAILABLE | AVAILABLE |
| LymphoTrack® Dx *TRB* Assays | AVAILABLE | PLEASE INQUIRE |
| **Research Use Only (RUO) Assays** | **MiSeq®** | **Ion S5/PGM™** |
| LymphoTrack® *IGHV* Somatic Hypermutation Assays | AVAILABLE | PLEASE INQUIRE |
| LymphoTrack® *IGH* FR1 Assays | AVAILABLE | AVAILABLE |
| LymphoTrack® *IGH* FR2 Assays | AVAILABLE | AVAILABLE |
| LymphoTrack® *IGH* FR3 Assays | AVAILABLE | AVAILABLE |
| LymphoTrack® *IGH* FR1/2/3 Assays | AVAILABLE | AVAILABLE |
| LymphoTrack® *IGK* Assays | AVAILABLE | AVAILABLE |
| LymphoTrack® *TRG* Assays | AVAILABLE | AVAILABLE |
| LymphoTrack® *TRB* Assay | AVAILABLE | PLEASE INQUIRE |

CE-marked assays are *in vitro* diagnostic products and are not available for sale or use within North America.
**Minimal residual disease (MRD) applications are currently for research use only.

# Gel and Capillary Electrophoresis Menu

Invivoscribe offers assays that can be analyzed using two conventional methods of fragment analysis: gel electrophoresis or capillary electrophoresis.

Gel electrophoresis kits offer a comparatively easy and inexpensive solution for clonality, translocation, and mutational testing and are often the method of choice for laboratories new to using these methods and techniques. PCR products are analyzed using non-denaturing polyacrylamide gels (PAGE) and often require a heteroduplex step for resolution of generated amplicons.

Capillary electrophoresis kits are supplied with fluorescently labeled primers, allowing the resulting PCR products to be analyzed on Applied Biosystems (ABI) platforms (e.g. 3130, 3500). Fragment analysis by capillary electrophoresis offers the ability to detect fragments with a high level of accuracy and analytical sensitivity and allows for greater sample throughput compared to gel detection methods. In addition, capillary electrophoresis detection often facilitates a more objective interpretation of results than gel-based detection.

The table below summarizes which detection methods are available for our clonality and translocation assays either as Research Use Only or CE-marked IVDs.

| CE-Marked IVD Assays | Gel | ABI |
|---|---|---|
| IdentiClone® *IGH* + *IGK* B-Cell Clonality Assay | AVAILABLE | AVAILABLE |
| IdentiClone® *IGH* Gene Clonality Assay | AVAILABLE | AVAILABLE |
| IdentiClone® *IGK* Gene Clonality Assay | AVAILABLE | AVAILABLE |
| IdentiClone® *IGL* Gene Clonality Assay | AVAILABLE | AVAILABLE |
| IdentiClone® *TCRB* + *TCRG* T-Cell Clonality Assay | AVAILABLE | AVAILABLE |
| IdentiClone® *TCRB* Gene Clonality Assay | AVAILABLE | AVAILABLE |
| IdentiClone® T-Cell Receptor Gamma Gene Rearrangement Assay 2.0 | NOT AVAILABLE | AVAILABLE |
| IdentiClone® *TCRG* Gene Clonality Assay | AVAILABLE | AVAILABLE |
| IdentiClone® *TCRD* Gene Clonality Assay | AVAILABLE | AVAILABLE |
| IdentiClone® *BCL1*/JH Translocation Assay | AVAILABLE | NOT AVAILABLE |
| IdentiClone® *BCL2*/JH Translocation Assay | AVAILABLE | NOT AVAILABLE |
| **Research Use Only (RUO) Assays** | **Gel** | **ABI** |
| *IGH* + *IGK* B-Cell Clonality Assay | AVAILABLE | AVAILABLE |
| *IGH* Gene Rearrangement Assay | AVAILABLE | AVAILABLE |
| *IGH* Gene Clonality Assay | AVAILABLE | AVAILABLE |
| *IGH* Somatic Hypermutation Assay v2.0 | AVAILABLE | AVAILABLE |
| *IGL* Gene Clonality Assay | AVAILABLE | AVAILABLE |
| *TCRB* + *TCRG* T-Cell Clonality Assay | AVAILABLE | AVAILABLE |
| *TCRB* Gene Clonality Assay | AVAILABLE | AVAILABLE |
| T-Cell Receptor Gamma Gene Rearrangement Assay | AVAILABLE | AVAILABLE |
| T-Cell Receptor Gamma Gene Rearrangement Assay 2.0 | NOT AVAILABLE | AVAILABLE |
| *TCRG* Gene Clonality Assay | AVAILABLE | AVAILABLE |
| *TCRD* Gene Clonality Assay | AVAILABLE | AVAILABLE |
| *BCL1*/JH Translocation Assay | AVAILABLE | NOT AVAILABLE |
| *BCL2*/JH Translocation Assay | AVAILABLE | NOT AVAILABLE |
| *BCL2*/JH t(14;18) Translocation Assay | AVAILABLE | NOT AVAILABLE |
| *BCR/ABL* t(9;22) Translocation Assay | AVAILABLE | AVAILABLE |
| *PML/RARA* t(15;17) Translocation Assay | AVAILABLE | AVAILABLE |

CE-marked assays are *in vitro* diagnostic products and are not available for sale or use within North America.

# Common Technical Support Questions

1. What sample types may be suitable for analysis with Invivoscribe Gel and Capillary assays?

   We recommend high-quality DNA for clonality testing with our assays. This can be extracted from frozen or fresh tissue, peripheral blood, bone marrow, skin biopsies, etc.

2. When should the recommended controls be run with our assays?

   The no template, positive, and negative controls should be included in every run for each target, per the product insert or instructions for use.

3. What is the purpose of the Specimen Control Size Ladder and Amplification Control master mix? What is the difference between these master mixes?

   The Specimen Control Size Ladder and Amplification Control master mixes are used as troubleshooting tools that allow you to determine if the quality and quantity of your DNA sample is suitable for use with our assays. The Specimen Control Size Ladder amplifies DNA at approximately 100, 200, 300, 400, and 600 base pairs; whereas, the Amplification Control amplifies DNA at 235 bp.

4. How should the master mix and controls be stored and thawed?

   The master mixes should be stored at -65 to -85 °C and should be thawed at room temperature and vortexed prior to use. If you intend to use master mixes multiple times, we recommend aliquoting the master mixes to minimize the number of freeze/thaw cycles. For the *FLT3* CDx Mutation Assay: Opened vials of master mixes stored frozen may incur up to 4 freeze thaw cycles. Opened vials of controls stored frozen may incur up to 8 freeze thaw cycles.

5. Where can more information about the primers used in our assays be found?

   Most primer information is proprietary to Invivoscribe and cannot be disclosed. We can, however, tell you the target area for the primers in each master mix, if you contact our support team by emailing support@invivoscribe.com or by calling +1 858-224-6600.

6. Which targets are recommended for the study of B-cell malignancies?

   The EuroClonality/BIOMED-2 Group has shown that combined testing of *IGH* and *IGK* achieves a clinical sensitivity of 99%. If purchasing these assays separately is cost prohibitive, our *IGH* + *IGK* Gene Clonality Assay (does not include *IGH* Tubes D and E) may be a feasible alternative option (see Figure 2 and Table 1 in *Leukemia* (2007) 21, 201-206). We also offer next-generation sequencing LymphoTrack® Assays for *IGH* and *IGK* for use with MiSeq® or Ion S5/PGM™ instruments. In addition, a high percentage of B-ALL patients have *TRG* rearrangements, which can be detected using our assays to detect *TRG* gene rearrangements.

7. What are the differences between our *IGH* Gene Rearrangement Assays and the *IGH* Gene Clonality Assays?

   The *IGH* Gene Rearrangement Assay was designed by Invivoscribe; whereas, the *IGH* Gene Clonality Assay was designed by the EuroClonality/BIOMED-2 Group. Both assays target the conserved IGH framework regions, Framework 1, Framework 2, and Framework 3. The *IGH* Gene Clonality Assay also targets incomplete $D_H$ - $J_H$ rearrangements. The *IGH* Gene Clonality Assay includes 33 reactions per master mix and the *IGH* Gene Rearrangement Assay includes 30 reactions per master mix.

8. What do *IGH* Tubes D and E target do and why are they challenging to interpret?

   Tubes D and E of our *IGH* Gene Clonality Assays target incomplete *IGH* $D_H$ - $J_H$ rearrangements. It is common to see known amplicons listed in the instructions for use in cases where a polyclonal background is absent (this is likely because these rearrangements are rare). Some of our customers are concerned by this, especially because there may be some samples that have robust germline amplification greater than the valid size range. We do not expect the germline amplification to outcompete true $D_H$ - $J_H$ rearrangements. PCR amplicons generated from germline templates are much larger than true $D_H$ - $J_H$ rearrangements. As a result, PCR products of germline amplifications are less robust when a specific target is present in samples.

9. Why does the polyclonal control produce a peak around 148 bp when amplified with *IGK* Tube A – 6FAM?

   The 148 bp peak is a result of the restricted repertoire of *IGK* and this peak commonly appears flanked by several smaller peaks on each side. It is still possible to have a true clonal rearrangement at this size in samples. If you suspect that this peak is clonal in one of your samples, we recommend following up with heteroduplex analysis. Alternatively, NGS-based LymphoTrack® and LymphoTrack® Dx Assays provide an easier interpretation for *IGK* and reduces the number of master mixes to just one reaction.

10. What T-cell receptor kits would you recommend to detect T-cell clonal rearrangements?

    Ideally, you should perform tests for *TRB*, *TRG*, and *TRD* to achieve the highest sensitivity. The EuroClonality/BIOMED-2 Group has shown that testing both *TRB* and *TRG* offers roughly the same sensitivity for the detection of T-cell malignancies as testing all three targets; however, they highly recommend testing all three assays in parallel to achieve optimal clinical sensitivity. *TRD* is especially useful in cases of suspected immature T-cell proliferations (see Figure 2 and Table 2 in *Leukemia* (2007) 21, 201-206). We also offer NGS kits for *TRG* for use with MiSeq® or Ion S5/PGM™ instruments and for *TRB* for use with MiSeq®.

11. What are the differences between the *TCRG* Gene Clonality Assay and the T-Cell Receptor Gamma Gene Rearrangement Assay 2.0?

    The *TCRG* Gene Clonality Assay was designed by the EuroClonality/ BIOMED-2 Group and consists of two master mixes. For polyclonal populations, four Gaussian distributions are generated. The T-Cell Receptor Gamma Gene Rearrangement Assay 2.0 was designed by Invivoscribe and it's performance was subsequently reviewed and validated by the EuroClonality/ BIOMED-2 Group. It targets all functional VH - JH rearrangements in a single master mix and produces smaller amplicons grouped under a single Gaussian distribution. This allows for easier interpretation and makes the assay more suitable for DNA extracted from FFPE tissue, which may consist of partially degraded DNA that would not amplify well with the larger valid size range of the *TCRG* Gene Clonality Assay.

12. What are the differences between the *IGH-BCL2* Translocation Assay and the *IGH-BCL2* t(14;18) Translocation Assay?

    The *IGH-BCL2* Translocation Assay was designed by the EuroClonality/BIOMED-2 Group and is available as either a CE-IVD or research use only assay whereas; the *IGH-BCL2* t(14;18) Translocation Assay was designed by Invivoscribe and is only available for research use only. Both of these assays target MBR and Mcr translocations, but the *IGH-BCL2* Translocation Assay also targets the translocations at the 3' Mbr. The *IGH-BCL2* t(14;18) Translocation Assay was designed as a nested PCR allowing greater sensitivities (1 clonal cell per 10,000 normal cells) to be achieved. The limit of detection of the *IGH-BCL2* Translocation Assay is 1 clonal cell per 100 normal cells. Lastly, the *IGH-BCL2* Translocation Assay includes 33 reactions, whereas the *IGH-BCL2* t(14;18) Translocation Assay includes 30 reactions.

13. Do you offer quantitative chromosome translocation (e.g., *BCR-ABL1*) controls?

    Our controls are validated for qualitative use, although our customers do successfully use them with quantitative assays. Unfortunately, we cannot guarantee their performance with any assay that was not designed by Invivoscribe.

14. Which capillary electrophoresis instruments are currently validated for use with our assay kits?

    Currently the capillary electrophoresis instruments Invivoscribe has validated include: ABI 3100 and 3130 series for all capillary electrophoresis detection assays. The ABI 310 and 3500 instrument series have also been validated for the majority of our capillary electrophoresis detection assays. We are not able to support using instruments not listed as validated in the instructions for use of our CE-IVD assays.

15. What are the recommended settings for my ABI instrument?

    Instruments should be calibrated with the DS-30 matrix standards (Dye set D) for the ABI 310, 3100, or 3130 instrument series. For the ABI 3500 sequencer series, we advise that you calibrate the instrument with DS-33 matrix standards. We also recommend using either POP-4 or POP-7 depending on which ABI instrument you are using. If your equipment supports POP-7, we recommend using this polymer as it can be utilized for both fragment analysis and sequencing; whereas, POP-4 can only be utilized for fragment analysis.

16. How should peaks outside the valid size range be interpreted when using assay kits?

    You should not interpret peaks outside of the valid size range; although, in theory, it is possible to have a true rearrangement fall outside this region. If you are concerned about a suspect peak, you may sequence your product for confirmation. Please note that samples should always be interpreted within the context of all available clinical information.

17. Is cell-free DNA (cfDNA) a suitable sample type for Invivoscribe LymphoTrack® or LymphoTrack® Dx Assays?

    The average size of cfDNA (~170 bps) makes it a suitable sample type to run with *IGH* FR3 master mixes. The use of cfDNA with *TRG* master mixes might be possible, but expected amplicon sizes generated with this assay are near the upper limits of the fragment lengths typically found with this sample type.

18. Is DNA extracted from FFPE tissue suitable to use with Invivoscribe LymphoTrack® or LymphoTrack® Dx Assays?

    To ensure DNA from challenging specimens is of sufficient quality and quantity to generate a valid result, samples may be tested with the Specimen Control Size Ladder master mix.

19. On which instruments can I use the LymphoTrack® and LymphoTrack® Dx Assays?

    We have different versions of our assays for the S5/PGM™ and MiSeq® instruments (LymphoTrack *TRB* is currently available only on MiSeq®). No other DNA sequencers (e.g. 454) are currently supported. Assays for the Ion S5/PGM™ and MiSeq® platforms differ slightly in terms of the total number of indices, etc., but both have similar benefits such as a one-step PCR reaction and included bioinformatics software.

20. How much DNA is needed for the LymphoTrack® and LymphoTrack® Dx Assays?

    50 ng of high-quality genomic DNA is required for the Ion S5/ PGM™ and MiSeq® LymphoTrack and LymphoTrack Dx Assays for clonality and somatic hypermutation applications.

21. Can I use a different library quantification method or kit?

We recommend using the KAPA™ kit for MiSeq® assays and either the 2100 Bioanalyzer® or the LabChip® GX for the Ion S5/PGM™ assays.

22. Will the LymphoTrack® or LymphoTrack® Dx analysis software work on my computer?

The software requires Microsoft Windows 7 (64-bit) and Excel 2007, 2010, or 2013 and will work with most desktop or laptop PCs. For specific requirements please refer to the software instructions for use.

23. Can I use the LymphoTrack® or LymphoTrack® Dx bioinformatics software with a different assay?

No, the software will only work with datasets obtained by our LymphoTrack and LymphoTrack Dx Assays.

24. What characters can I use when naming my samples and the file pathways? What types of files are accepted by the LymphoTrack® and LymphoTrack® Dx Software - MiSeq®?

Our software only recognizes file names and pathways that contain the following characters (A-Z, a-z, 0-9, . (dot), _ (underscore), - (hyphen)). In addition, spaces in the pathname for the data files or software (pathnames include file folders and file names) should be avoided. If the software encounters a character that is not listed above or extra spaces, an error message may be generated. Furthermore, the software is only compatible with adaptor-trimmed fastq.gz files that are generated by the MiSeq® Reporter Software when the MiSeq® instrument is used. An example of the naming format that the MiSeq® Reporter uses: SampleName_S1_L001_R1_001.fastq.gz and SampleName_S1_L001_R2_001.fastq.gz.

25. Do the Invivoscribe MiSeq® indices correspond to the Illumina® indices?

The indices included in our MiSeq® master mixes follow Illumina®'s TruSeq LT nomenclature. For instance, *IGH* FR1 MiSeq® 01 corresponds to A001. Information for the other indices can be found in the instructions for use on how to set up the MiSeq® Sample Sheet to detect the appropriate indices.

26. Why am I getting a low percent passing filter and Q30 score?

Low Q30 and percent passing filter (%PF) scores could be an indication that the flow cell is overloaded. If this is suspected, verify your amplicon and library calculations and quantifications are correct. Low run metrics can also be attributed to many additional factors including poor quality DNA, contamination, flow cell or instrument issues, etc. Please refer to your Illumina MiSeq® user guides and contact Illumina® Support.

27. Why is the same $V_H$-$J_H$ rearrangement combination and sequence shared by two groups of reads, one of which is several bases shorter than the other when looking at the Read Summary tab of the excel document created by the LymphoTrack® Visualization Tool?

Our software was designed to list every unique sequence separately in order for the customer to see all of the data and make their own determination on how to interpret it. The several base pair difference can be due to a number of factors including amplification errors and sequencing errors. It could also be a result of similarities between some of the primer sequences that were designed to ensure maximum coverage. We also include a Merged Read Summary report for your reference that combines sequences that only differ by 1 or 2 basepairs.

28. Do I need to perform an adapter ligation prior to sequencing my products?

Performing an adapter ligation is not needed. The primers included in our LymphoTrack and LymphoTrack Dx master mixes already include the appropriate index barcodes and adapter sequences. After PCR amplification, you will be able to proceed with amplicon purification, amplicon quantification, library pooling, and sequencing.

29. If the LymphoTrack® or LymphoTrack® Dx software generated an error, what information should I submit to Technical Support?

Please submit the *.txt Log file that should have been created by the software in the output folder, a screenshot of the sample directory, and the Lot Number of the software CD you are using to support@invivoscribe.com.

30. Are controls provided with the kits? Can you purchase additional controls? How are they supplied?

Each kit contains the necessary positive and negative controls required to perform the assay; additional controls may also be purchased separately. Single-tube DNA controls are provided as 100 µL aliquots of 200 µg/mL in 1/10 TE Buffer, 50 µL aliquots of 50 µg/mL in 1/10 TE Buffer, and 45 µL aliquots of 15 µg/mL in 1/10 TE Buffer. Single-tube RNA controls are provided as 100 µL aliquots of 400 µg/ml in RNAse free in glass distilled water.

31. What are the differences between dilution sets, sensitivity panels, and proficiency panels?

31a. RNA Dilution Sets
BCR/ABL b3a2 (Cat# 4-085-0210), BCR/ABL b2a2 (Cat# 4-085-0310), and BCR/ABL e1a2 (Cat# 4-085-0110). These sets contain six tubes: 100% negative control RNA and volume to volume (v/v) dilutions ($10^{-1}$, $10^{-2}$, $10^{-3}$, $10^{-4}$, and $10^{-5}$) of the positive control RNA into the negative control RNA (IVS-0048). The RNA Dilution Sets are supplied at a concentration of 400 µg/mL, and each tube contains 50 µL. These dilution sets may be used to establish a standard reference curve, as proficiency controls, as sensitivity controls for specific target assays, and as routine testing controls for cDNA synthesis, amplification and detection.

31b. RNA Sensitivity Panels
These panels consist of seven tubes: 100% positive control RNA and v/v dilutions ($10^{-1}$, $10^{-2}$, $10^{-3}$, $10^{-4}$, $10^{-5}$, and $10^{-6}$) of the positive control RNA into the negative control RNA (IVS-0035). The RNA Sensitivity Panels are supplied at a concentration of 400 µg/mL, and each tube contains 100 µL. The RNA Sensitivity Panels may be used as sensitivity controls for specific target assays, and as routine testing controls for cDNA synthesis, amplification and detection.

31c. DNA Sensitivity Panels
Consist of six tubes: 100% clonal DNA and v/v dilutions of the clonal DNA into negative polyclonal DNA (IVS-0000) to make 30%, 20%, 10%, 5%, and 1% dilutions. The DNA Sensitivity Panels are supplied at a concentration of 200 µg/mL and each tube contains 100 µL. The DNA Sensitivity Panels may be used as sensitivity controls for specific target assays.

31d. RNA Proficiency Panel
The proficiency panel for BCR-ABL1 t(9;22) can be used as a sensitivity control for specific target assays, and as routine testing controls for cDNA synthesis, amplification and detection. It consists of ten tubes: 100% positive control RNA and v/v dilutions ($10^{-2}$ and $10^{-4}$) of IVS-0003, IVS-0011 and IVS-0032. It also includes BCR-ABL1 Negative Clonal Control RNA (IVS-0035).

# Poster Abstracts

**Evaluation of an Alternative Fragmentation Method in High Throughput NGS Sample Testing of Minimal Residual Disease in Hematological Malignancies**
Poster Presented at: Association of Molecular Pathology 2019 Annual Meeting. 2019 Nov. 7-9. Baltimore, MD, USA.

**A Next-Generation-Sequencing Based Analysis of Clonality across 39 Subjects Treated for Lymphoproliferative Disorders Reveals Matching Clones in the Diverse *IGH* Locus**
Poster Presented at: Association of Molecular Pathology 2019 Annual Meeting. 2019 Nov. 7-9. Baltimore, MD, USA.

**Comparing DNA Extraction Methods for the LymphoTrack IVD *TRG* Assay**
Poster Presented at: Association of Molecular Pathology 2019 Annual Meeting. 2019 Nov. 7-9. Baltimore, MD, USA.

**Comparing Minimal Residual Disease Detection in Multiple Myeloma using NGS-Based LymphoTrack® Assays and Flow Cytometry**
Poster Presented at: Association of Molecular Pathology Global 2019 Annual Meeting. 2019 May 16-18. Hong Kong, CN.

**LymphoTrack® Low Positive Control and LymphoQuant® Internal Control for MiSeq® and Ion S5/PGM™ LymphoTrack Assays**
Poster Presented at: Association of Molecular Pathology Global 2019 Annual Meeting. 2019 May 16-18. Hong Kong, CN.

**Validation of an NGS Based Assay for Monitoring *FLT3* ITD and TKD Variants in AML Subjects**
Poster Presented at: Association of Molecular Pathology Global 2019 Annual Meeting. 2019 May 16-18. Hong Kong, CN.

**Minimal Residual Disease in AML can be Monitored Utilizing Cell-Free DNA**
Poster Presented at: Association of Molecular Pathology 2018 Annual Meeting. 2018 Nov. 1-3. San Antonio, TX, USA.

**Detection of Clonal Rearrangements in Multiple Myeloma Sample using LymphoTrack® Assays**
Poster Presented at: Association of Molecular Pathology 2018 Annual Meeting. 2018 Nov. 1-3. San Antonio, TX, USA.

**Genetic Heterogeneity and Stratification of AML Samples with *NPM1* Mutation Detected by the MyAML® NGS Test**
Poster presented at: Association for Molecular Pathology 2017 Annual Meeting. 2017 November 16-18, Salt Lake City, UT, USA.

**Clonality Detection Using Next-Generation Sequencing and Capillary Electrophoresis Methods in Suspect Lymphoproliferative Samples**
Poster presented at: Association for Molecular Pathology 2017 Annual Meeting. 2017 November 16-18, Salt Lake City, UT, USA.

**Validation of the LeukoStrat® CDx *FLT3* Mutation Assay: Used to Detect both Internal Tandem Duplication (ITD) and Tyrosine Kinase Domain (TKD) Mutations and Response to Midostaurin in 1058 Patients with AML**
Poster presented at: Association for Molecular Pathology 2017 Annual Meeting. 2017 November 16-18, Salt Lake City, UT, USA.

**Multiple Highly Concordant Assays Facilitate Analyses of Clinical Samples at Different Scales and Sensitivities**
Poster presented at: Association for Molecular Pathology 2017 Annual Meeting. 2017 November 16-18, Salt Lake City, UT, USA.

**Detection of Clonal *TRG* and *TRB* Gene Rearrangements Using Next-Generation Sequencing**
Poster presented at: Association for Molecular Pathology 2017 Annual Meeting. 2017 November 16-18, Salt Lake City, UT, USA.

**Analysis and Characterization of Hematologic Cancers Using a Comprehensive NGS Panel Comprised of DNA and RNA Baits Targeting 704 Genes**
Poster presented at: 22nd Congress of the European Hematology Association. 2017 June 22-25. Madrid, Spain.

**Next Generation Sequencing Targeted Panel for Minimal Residual Disease Monitoring in Acute Myeloid Leukemia**
Poster presented at: 22nd Congress of the European Hematology Association. 2017 June 22-25. Madrid, Spain.

**Detection of Clonality in Clinical Specimens from Suspected B-Cell Malignancies Using Comprehensive *IGH* Lymphotrack® MiSeq® and PGM® Assays**
Poster presented at: 22nd Congress of the European Hematology Association. 2017 June 22-25. Madrid, Spain.

**Small Customizable NGS Based Target Capture Panels Detect Variants in Clinical Specimens at Frequencies as Low as 0.5%**
Poster presented at: 22nd Congress of the European Hematology Association. 2017 June 22-25. Madrid, Spain.

**Detecting B-Cell Clonality in Clinical Samples using a Comprehensive NGS LymphoTrack Dx® *IGH* FR1/2/3 Assay**
Poster presented at: Association for Molecular Pathology Global 2017 Meeting. 2017 April 3-5. Berlin, Germany.

**Assessment of Minimal Residual Disease in Patients with Acute Myeloid Leukemia by Monitoring *FLT3* and *NPM1* Mutations**
Poster presented at: Association for Molecular Pathology Global 2017 Meeting. 2017 April 3-5. Berlin, Germany.

**A Precision Medicine Approach Incorporating Both Molecular and In Vitro Functional Data to Treat Patients with Relapsed/Refractory Acute Myeloid Leukemia**
Poster Presented at: 58th Annual American Society for Hematology Meeting. 2016 Dec. 3-6. San Diego, CA, USA.

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**Precision Medicine Assays Detect Novel Targetable *FLT3* Fusions Amenable to Therapeutic Intervention in a Patient with Refractory Acute Myeloid Leukemia**
Poster Presented at: 58th Annual American Society for Hematology Meeting. 2016 Dec. 3-6. San Diego, CA, USA.

**Next-Generation Sequencing of *FLT3/ITD* for Minimal Residual Disease Monitoring in Leukemia Patients**
Poster Presented at: Association for Molecular Pathology 2016 Annual Meeting. November 10-12, 2016 at the Charlotte Convention Center in Charlotte, NC, USA.

**Next-Generation Sequencing of *NPM1* for Minimal Residual Disease Monitoring in Leukemia Patients**
Poster Presented at: Association for Molecular Pathology 2016 Annual Meeting. November 10-12, 2016 at the Charlotte Convention Center in Charlotte, NC, USA.

**Detection of Clonal Immunoglobulin and T-Cell Receptor Gene Rearrangements in Acute Myeloid Leukemia**
Poster Presented at: Association for Molecular Pathology 2016 Annual Meeting. November 10-12, 2016 at the Charlotte Convention Center in Charlotte, NC, USA.

**Development of a Comprehensive *IGH* NGS Assay for Detecting Suspected B-Cell Clonality**
Poster Presented at: Association for Molecular Pathology 2016 Annual Meeting. November 10-12, 2016 at the Charlotte Convention Center in Charlotte, NC, USA.

**Detection of Minimal Residual Disease in *FLT3/ITD* AML**
Poster presented at: American Society of Clinical Oncology 2016 Annual Meeting. June 3-7, 2016 at McCormick Place in Chicago, Illinois, USA.

**Development of LymphoTrack® Bioinformatics Methods: Clonality Testing, Somatic Hypermutation, and Minimal Residual Disease**
Poster Presented at: Association for Molecular Pathology 2015 Annual Meeting. 2015 Nov 4-8. Austin, TX, USA.

**Detection of Clonal *TRG* Gene Rearrangements Shows Improved Sensitivity and Positive Predictive Value Compared to Fragment Analysis Using BIOMED-2 Primers and Capillary Electrophoresis**
Poster Presented at: Association for Molecular Pathology 2015 Annual Meeting. 2015 Nov 4-8. Austin, TX, USA.

**Development of an NGS Assay for *IGK* that can be Combined with *IGH* for Identifying Clonal Populations in Lymphoid Malignancies**
Poster Presented at: 20th Congress of the European Hematology Association. 2015 June 11-14. Vienna, Austria.

**Clinical Assessment of Chronic Lymphocytic Leukemia (CLL) samples for Somatic Hypermutation Status by Next-Generation Sequencing and Sanger Sequencing**
Poster Presented at: 20th Congress of the European Hematology Association. 2015 June 11-14. Vienna, Austria.

**Somatic Hypermutation and V-J Gene Usage for CLL Prognosis: Evaluating Data from MiSeq® NGS vs. PCR-Sanger Sequencing Approaches**
Poster Presented at: 56th Annual American Society for Hematology Meeting. 2014 Dec. 6-9. San Francisco, CA, USA.

**International Validation of a Harmonized NGS PGM™ Assay at Clinical Labs in the US and the EU**
Poster Presented at: Association for Molecular Pathology 2014 Annual Meeting. 2014 Nov 13-15. National Harbor, MD, USA.

**Performance of an *IGH* Somatic Hypermutation Assay with Associated LymphoTrack® Bioinformatics Developed for the MiSeq® NGS Platform**
Poster Presented at: 19th Congress of the European Hematology Association. 2014 June 12-15. Milan, Italy.

**Concordance in *IGH* & *TRG* Clonality Testing: Comparison of Data Generated Using the MiSeq® & PGM™ Platforms**
Poster Presented at: 19th Congress of the European Hematology Association. 2014 June 12-15. Milan, Italy.

**Combined *TRG* and *IGH* Clonality Testing on the PGM™ Using LymphoTrack® Reagents & Bioinformatics**
Poster Presented at: 19th Congress of the European Hematology Association. 2014 June 12-15. Milan, Italy.

**Identifying and Monitoring *IGH* Clonality Using Massively Parallel Sequencing and Associated Bioinformatics**
Poster Presented at: Association for Molecular Pathology 2013 Annual Meeting. 2013 Nov. 15-16. Phoenix, AZ, USA.

**Detecting Minimal Residual Disease Using a Massively Parallel Sequencing *TCRG* Assay**
Poster Presented at: Association for Molecular Pathology 2013 Annual Meeting. 2013 Nov. 15-16. Phoenix, AZ, USA.

**Identifying and Monitoring *TCRG* Clonality Using Massively Parallel Sequencing and Associated Bioinformatics**
Poster Presented at: 18th Congress of the European Hematology Association. 2013 June 13-16. Stockholm, Sweden.

# Product List by Catalog Number

## Research Use Only Assays
## Capillary & Gel Fragment Analysis

1-100-0010 *IGH + IGK* B-Cell Clonality Assay – Gel Detection
1-100-0020 *IGH + IGK* B-Cell Clonality Assay MegaKit – Gel Detection
1-100-0031 *IGH + IGK* B-Cell Clonality Assay – ABI Fluorescence Detection
1-100-0041 *IGH + IGK* B-Cell Clonality Assay MegaKit – ABI Fluorescence Detection
1-101-0010 *IGH* Gene Rearrangement Assay – Gel Detection
1-101-0020 *IGH* Gene Clonality Assay – Gel Detection
1-101-0030 *IGH* Gene Rearrangement Assay MegaKit – Gel Detection
1-101-0040 *IGH* Gene Clonality Assay MegaKit – Gel Detection
1-101-0051 *IGH* Gene Rearrangement Assay – ABI Fluorescence Detection
1-101-0061 *IGH* Gene Clonality Assay – ABI Fluorescence Detection
1-101-0071 *IGH* Gene Rearrangement Assay MegaKit – ABI Fluorescence Detection
1-101-0081 *IGH* Gene Clonality Assay MegaKit – ABI Fluorescence Detection
1-102-0020 *IGK* Gene Clonality Assay – Gel Detection
1-102-0021 *IGK* Gene Clonality Assay – ABI Fluorescence Detection
1-102-0030 *IGK* Gene Clonality Assay MegaKit – Gel Detection
1-102-0031 *IGK* Gene Clonality Assay MegaKit – ABI Fluorescence Detection
1-103-0010 *IGL* Gene Clonality Assay – Gel Detection
1-103-0011 *IGL* Gene Clonality Assay – ABI Fluorescence Detection
1-103-0020 *IGL* Gene Clonality Assay MegaKit – Gel Detection
1-103-0021 *IGL* Gene Clonality Assay MegaKit – ABI Fluorescence Detection
1-200-0010 *TCRB + TCRG* T-Cell Clonality Assay – Gel Detection
1-200-0011 *TCRB + TCRG* T-Cell Clonality Assay – ABI Fluorescence Detection
1-200-0020 *TCRB + TCRG* T-Cell Clonality Assay MegaKit – Gel Detection
1-200-0021 *TCRB + TCRG* T-Cell Clonality Assay MegaKit – ABI Fluorescence Detection
1-205-0010 *TCRB* Gene Clonality Assay – Gel Detection
1-205-0011 *TCRB* Gene Clonality Assay – ABI Fluorescence Detection
1-205-0020 *TCRB* Gene Clonality Assay MegaKit – Gel Detection
1-205-0021 *TCRB* Gene Clonality Assay MegaKit – ABI Fluorescence Detection
1-206-0010 *TCRD* Gene Clonality Assay – Gel Detection
1-206-0011 *TCRD* Gene Clonality Assay – ABI Fluorescence Detection
1-206-0020 *TCRD* Gene Clonality Assay MegaKit – Gel Detection
1-206-0021 *TCRD* Gene Clonality Assay MegaKit – ABI Fluorescence Detection
1-207-0010 T-Cell Receptor Gamma Gene Rearrangement Assay – Gel Detection
1-207-0020 *TCRG* Gene Clonality Assay – Gel Detection
1-207-0021 *TCRG* Gene Clonality Assay – ABI Fluorescence Detection
1-207-0030 T-Cell Receptor Gamma Gene Rearrangement Assay MegaKit – Gel Detection
1-207-0040 *TCRG* Gene Clonality Assay MegaKit – Gel Detection
1-207-0041 *TCRG* Gene Clonality Assay MegaKit – ABI Fluorescence Detection
1-207-0051 T-Cell Receptor Gamma Gene Rearrangement Assay – ABI Fluorescence Detection
1-207-0071 T-Cell Receptor Gamma Gene Rearrangement Assay MegaKit – ABI Fluorescence Detection
1-207-0101 T-Cell Receptor Gamma Gene Rearrangement Assay 2.0 – ABI Fluorescence Detection
1-207-0111 T-Cell Receptor Gamma Gene Rearrangement Assay 2.0 MegaKit – ABI Fluorescence Detection
1-308-0010 *BCL1/J*H Translocation Assay – Gel Detection
1-308-0020 *BCL1/J*H Translocation Assay MegaKit – Gel Detection
1-309-0010 *BCL2/J*H t(14;18) Translocation Assay – Gel Detection
1-309-0020 *BCL2/J*H Translocation Assay – Gel Detection
1-309-0030 *BCL2/J*H t(14;18) Translocation Assay MegaKit – Gel Detection
1-309-0040 *BCL2/J*H Translocation Assay MegaKit – Gel Detection
1-310-0010 *BCR/ABL* t(9;22) Translocation Assay – Gel Detection
1-310-0020 *BCR/ABL* t(9;22) Translocation Assay MegaKit – Gel Detection
1-310-0031 *BCR/ABL* t(9;22) Translocation Assay – ABI Fluorescence Detection
1-310-0041 *BCR/ABL* t(9;22) Translocation Assay MegaKit – ABI Fluorescence Detection
1-311-0010 *PML/RARa* t(15;17) Translocation Assay – Gel Detection
1-311-0011 *PML/RARa* t(15;17) Translocation Assay – ABI Fluorescence Detection
1-311-0020 *PML/RARa* t(15;17) Translocation Assay MegaKit – Gel Detection
1-311-0021 *PML/RARa* t(15;17) Translocation Assay MegaKit – ABI Fluorescence Detection
1-412-0010 *FLT3* Mutation Assay - Gel Detection
1-412-0020 *FLT3* Mutation Assay MegaKit - Gel Detection
1-412-0031 *FLT3* Mutation Assay - ABI Fluorescence Detection
1-412-0041 *FLT3* Mutation Assay MegaKit - ABI Fluorescence Detection

## Master Mixes

2-096-0010 Amplification Control Master Mix – Unlabeled
2-096-0011 Amplification Control Master Mix – 6FAM
2-096-0020 Specimen Control Size Ladder – Unlabeled
2-096-0021 Specimen Control Size Ladder – 6FAM
2-101-0010 *IGH* Tube A – Unlabeled
2-101-0011 *IGH* Tube A – 6FAM
2-101-0020 *IGH* Tube B – Unlabeled
2-101-0030 *IGH* Tube C – Unlabeled
2-101-0031 *IGH* Tube C – HEX
2-101-0040 *IGH* Tube D – Unlabeled
2-101-0041 *IGH* Tube D – HEX
2-101-0050 *IGH* Tube E – Unlabeled
2-101-0051 *IGH* Tube E – 6FAM
2-101-0060 *IGH* Framework 1 (FR1) – Unlabeled
2-101-0061 *IGH* Framework 1 (FR1) – 6FAM
2-101-0070 *IGH* Framework 2 (FR2) – Unlabeled
2-101-0080 *IGH* Framework 3 (FR3) – Unlabeled
2-101-0081 *IGH* Framework 3 (FR3) – HEX
2-101-0091 *IGH* Framework 2 (FR2) – 6FAM
2-101-0101 *IGH* Tube B – 6FAM
2-101-0170 Hypermutation Mix 1 v2.0 – Unlabeled
2-101-0171 Hypermutation Mix 1 v2.0 – 6FAM
2-101-0180 Hypermutation Mix 2 v2.0 – Unlabeled
2-101-0181 Hypermutation Mix 2 v2.0 – 6FAM
2-102-0010 *IGK* Tube A – Unlabeled
2-102-0011 *IGK* Tube A – 6FAM
2-102-0020 *IGK* Tube B – Unlabeled
2-102-0021 *IGK* Tube B – 6FAM
2-103-0010 *IGL* Tube – Unlabeled
2-103-0011 *IGL* Tube – 6FAM
2-205-0010 *TCRB* Tube A – Unlabeled
2-205-0011 *TCRB* Tube A – 6FAM & HEX
2-205-0020 *TCRB* Tube B – Unlabeled
2-205-0021 *TCRB* Tube B – 6FAM
2-205-0030 *TCRB* Tube C – Unlabeled
2-205-0031 *TCRB* Tube C – 6FAM & HEX
2-206-0010 *TCRD* Tube – Unlabeled
2-206-0011 *TCRD* Tube – 6FAM & HEX
2-207-0010 T-Cell Receptor Gamma Mix 1 – Unlabeled
2-207-0020 T-Cell Receptor Gamma Mix 2 – Unlabeled
2-207-0021 T-Cell Receptor Gamma Mix 2 – HEX
2-207-0030 *TCRG* Tube A – Unlabeled
2-207-0031 *TCRG* Tube A – 6FAM & HEX
2-207-0040 *TCRG* Tube B – Unlabeled
2-207-0041 *TCRG* Tube B – 6FAM & HEX
2-207-0071 T-Cell Receptor Gamma Mix 1 – 6FAM
2-207-0091 *TCRG* – 6FAM
2-308-0010 *BCL1/J*H Tube – Unlabeled
2-309-0010 *BCL2/J*H t(14;18) (Mbr) Mix 1b – Unlabeled
2-309-0020 *BCL2/J*H t(14;18) (mcr) Mix 2b – Unlabeled
2-309-0030 *BCL2/J*H t(14;18) (Mbr) Mix 1a – Unlabeled
2-309-0040 *BCL2/J*H t(14;18) (mcr) Mix 2a – Unlabeled
2-309-0050 *BCL2/J*H Tube A – Unlabeled
2-309-0060 *BCL2/J*H Tube B – Unlabeled
2-309-0070 *BCL2/J*H Tube C – Unlabeled
2-310-0010 *BCR/ABL* t(9;22) Mix 1a – Unlabeled
2-310-0020 *BCR/ABL* t(9;22) Mix 2a – Unlabeled
2-310-0030 *BCR/ABL* t(9;22) Mix 3a – Unlabeled
2-310-0040 *BCR/ABL* t(9;22) Mix 1b – Unlabeled
2-310-0041 *BCR/ABL* t(9;22) Mix 1b – HEX
2-310-0050 *BCR/ABL* t(9;22) Mix 2b – Unlabeled
2-310-0051 *BCR/ABL* t(9;22) Mix 2b – HEX
2-310-0060 *BCR/ABL* t(9;22) Mix 2c – Unlabeled
2-310-0061 *BCR/ABL* t(9;22) Mix 2c – HEX
2-310-0070 *BCR/ABL* t(9;22) Mix 3b – Unlabeled
2-310-0071 *BCR/ABL* t(9;22) Mix 3b – 6FAM
2-310-0080 *BCR/ABL* t(9;22) Mix 3c – Unlabeled
2-310-0081 *BCR/ABL* t(9;22) Mix 3c – 6FAM
2-310-0090 *BCR/ABL* t(9;22) Mix 3d – Unlabeled
2-310-0101 *BCR/ABL* t(9;22) Mix 3d – 6FAM
2-311-0011 *PML/RARa* t(15;17) Mix 1 – HEX
2-311-0031 *PML/RARa* t(15;17) Mix 2b – HEX
2-311-0041 *PML/RARa* t(15;17) Mix 2c – HEX

## *BCR/ABL* RNA Dilution Sets

4-085-0110 *BCR/ABL* e1a2 RNA Dilution Set
4-085-0210 *BCR/ABL* b3a2 RNA Dilution Set
4-085-0310 *BCR/ABL* b2a2 RNA Dilution Set

## DNA Sensitivity Panels

4-086-0040 Sensitivity Panel – IVS-0004 Clonal Control DNA
4-086-0070 Sensitivity Panel – IVS-0007 Clonal Control DNA
4-086-0090 Sensitivity Panel – IVS-0009 Clonal Control DNA
4-086-0100 Sensitivity Panel – IVS-0010 Clonal Control DNA
4-086-0190 Sensitivity Panel – IVS-0019 Clonal Control DNA
4-086-0210 Sensitivity Panel – IVS-0021 Clonal Control DNA
4-086-0300 Sensitivity Panel – IVS-0030 Clonal Control DNA

## RNA Sensitivity Panels

4-087-0030 Sensitivity Panel – IVS-0003 Clonal Control RNA
4-087-0110 Sensitivity Panel – IVS-0011 Clonal Control RNA
4-087-0150 Sensitivity Panel – IVS-0015 Clonal Control RNA
4-087-0200 Sensitivity Panel – IVS-0020 Clonal Control RNA
4-087-0320 Sensitivity Panel – IVS-0032 Clonal Control RNA

## Cell Line DNA Controls

R-088-0230 *FLT3* ITD Positive Control DNA - GPR
R-088-0240 *FLT3* TKD Positive Control DNA - GPR
R-088-0250 *FLT3* Extraction Control - GPR
4-088-0008 *IGH* SHM Positive Control DNA
4-088-0010 IVS-0001 Clonal Control DNA
4-088-0098 LymphoTrack® B-cell Low Positive Control
4-088-0108 LymphoTrack® T-cell Low Positive Control
4-088-0118 LymphoQuant® B-cell Internal Control
4-088-0128 LymphoQuant® T-cell Internal Control
4-088-0190 IVS-0004 Clonal Control DNA
4-088-0210 20% IVS-0004 Clonal Control DNA
4-088-0220 10% IVS-0004 Clonal Control DNA
4-088-0230 5% IVS-0004 Clonal Control DNA
4-088-0370 IVS-0007 Clonal Control DNA
4-088-0390 20% IVS-0007 Clonal Control DNA
4-088-0400 10% IVS-0007 Clonal Control DNA
4-088-0410 5% IVS-0007 Clonal Control DNA
4-088-0420 1% IVS-0007 Clonal Control DNA
4-088-0430 IVS-0008 Clonal Control DNA
4-088-0470 5% IVS-0008 Clonal Control DNA
4-088-0480 1% IVS-0008 Clonal Control DNA
4-088-0490 IVS-0009 Clonal Control DNA
4-088-0500 30% IVS-0009 Clonal Control DNA
4-088-0510 20% IVS-0009 Clonal Control DNA
4-088-0520 10% IVS-0009 Clonal Control DNA
4-088-0530 5% IVS-0009 Clonal Control DNA
4-088-0540 1% IVS-0009 Clonal Control DNA
4-088-0550 IVS-0010 Clonal Control DNA
4-088-0560 30% IVS-0010 Clonal Control DNA
4-088-0580 10% IVS-0010 Clonal Control DNA
4-088-0590 5% IVS-0010 Clonal Control DNA
4-088-0730 IVS-0013 Clonal Control DNA
4-088-1090 IVS-0019 Clonal Control DNA
4-088-1100 30% IVS-0019 Clonal Control DNA
4-088-1110 20% IVS-0019 Clonal Control DNA
4-088-1120 10% IVS-0019 Clonal Control DNA
4-088-1130 5% IVS-0019 Clonal Control DNA
4-088-1140 1% IVS-0019 Clonal Control DNA
4-088-1210 IVS-0021 Clonal Control DNA
4-088-1220 30% IVS-0021 Clonal Control DNA
4-088-1230 20% IVS-0021 Clonal Control DNA
4-088-1240 10% IVS-0021 Clonal Control DNA
4-088-1250 5% IVS-0021 Clonal Control DNA
4-088-1260 1% IVS-0021 Clonal Control DNA
4-088-1390 IVS-0024 Clonal Control DNA
4-088-1430 5% IVS-0024 Clonal Control DNA
4-088-1690 IVS-0029 Clonal Control DNA
4-088-1700 30% IVS-0029 Clonal Control DNA
4-088-1730 5% IVS-0029 Clonal Control DNA
4-088-1750 IVS-0030 Clonal Control DNA
4-088-1760 30% IVS-0030 Clonal Control DNA
4-088-1770 20% IVS-0030 Clonal Control DNA
4-088-1780 10% IVS-0030 Clonal Control DNA
4-088-1790 5% IVS-0030 Clonal Control DNA
4-088-1800 1% IVS-0030 Clonal Control DNA
4-088-1810 IVS-0031 Clonal Control DNA
4-088-1840 10% IVS-0031 Clonal Control DNA
4-088-1860 1% IVS-0031 Clonal Control DNA

## Cell Line RNA Controls

4-089-0100 IVS-0002 Clonal Control RNA
4-089-0190 IVS-0003 Clonal Control RNA
4-089-0200 $10^{-1}$ IVS-0003 Clonal Control RNA
4-089-0210 $10^{-2}$ IVS-0003 Clonal Control RNA
4-089-0220 $10^{-3}$ IVS-0003 Clonal Control RNA
4-089-0230 $10^{-4}$ IVS-0003 Clonal Control RNA
4-089-0240 $10^{-5}$ IVS-0003 Clonal Control RNA
4-089-0250 $10^{-6}$ IVS-0003 Clonal Control RNA
4-089-0910 IVS-0011 Clonal Control RNA
4-089-0920 $10^{-1}$ IVS-0011 Clonal Control RNA
4-089-0930 $10^{-2}$ IVS-0011 Clonal Control RNA
4-089-0940 $10^{-3}$ IVS-0011 Clonal Control RNA
4-089-0950 $10^{-4}$ IVS-0011 Clonal Control RNA
4-089-0960 $10^{-5}$ IVS-0011 Clonal Control RNA
4-089-1270 IVS-0015 Clonal Control RNA
4-089-1720 IVS-0020 Clonal Control RNA
4-089-1730 $10^{-1}$ IVS-0020 Clonal Control RNA
4-089-1740 $10^{-2}$ IVS-0020 Clonal Control RNA
4-089-1750 $10^{-3}$ IVS-0020 Clonal Control RNA
4-089-1760 $10^{-4}$ IVS-0020 Clonal Control RNA
4-089-2800 IVS-0032 Clonal Control RNA
4-089-2810 $10^{-1}$ IVS-0032 Clonal Control RNA
4-089-2820 $10^{-2}$ IVS-0032 Clonal Control RNA
4-089-2830 $10^{-3}$ IVS-0032 Clonal Control RNA
4-089-2840 $10^{-4}$ IVS-0032 Clonal Control RNA
4-089-2850 $10^{-5}$ IVS-0032 Clonal Control RNA
4-089-2860 $10^{-6}$ IVS-0032 Clonal Control RNA
4-089-3070 IVS-0035 Clonal Control RNA

## Plasmid DNA Control

4-090-0070 IVS-P002 Clonal Control DNA

## Tissue DNA Control

4-092-0010 IVS-0000 Polyclonal Control DNA

## RNA Proficiency Panel

4-310-0100 Proficiency Panel for *BCR/ABL* t(9;22) Translocations

## Somatic Hypermutation Sanger Sequencing Assays

5-101-0030 *IGH* Somatic Hypermutation Assay v2.0 – Gel Detection
5-101-0031 *IGH* Somatic Hypermutation Assay v2.0 – ABI Fluorescence Detection
5-101-0040 *IGH* Somatic Hypermutation Assay v2.0 MegaKit – Gel Detection
5-101-0041 *IGH* Somatic Hypermutation Assay v2.0 MegaKit – ABI Fluorescence

## ABI Reagents

6-098-0051 HI-Deionized Formamide with ROX Size Standard (ABI 310)
6-098-0061 HI-Deionized Formamide with ROX Size Standard (ABI 3100)

## Next-Generation Sequencing CE-IVD LymphoTrack® Dx Assays

9-121-0059 LymphoTrack® Dx *IGHV* Leader Somatic Hypermutation Assay Kit A – MiSeq®
9-121-0069 LymphoTrack® Dx *IGHV* Leader Somatic Hypermutation Assay Panel – MiSeq®
9-121-0129 LymphoTrack® Dx *IGH* FR1/2/3 Assay Kit A – MiSeq®
9-121-0139 LymphoTrack® Dx *IGH* FR1/2/3 Assay Panel – MiSeq®
9-121-0009 LymphoTrack® Dx *IGH* FR1 Assay Kit A – MiSeq®
9-121-0039 LymphoTrack® Dx *IGH* FR1 Assay Panel – MiSeq®
9-121-0089 LymphoTrack® Dx *IGH* FR2 Assay Kit A – MiSeq®
9-121-0099 LymphoTrack® Dx *IGH* FR2 Assay Panel – MiSeq®
9-121-0109 LymphoTrack® Dx *IGH* FR3 Assay Kit A – MiSeq®
9-121-0119 LymphoTrack® Dx *IGH* FR3 Assay Panel – MiSeq®
9-121-0057 LymphoTrack® Dx *IGH* FR1/2/3 Assay – PGM™
9-121-0007 LymphoTrack® Dx *IGH* FR1 Assay – PGM™
9-121-0037 LymphoTrack® Dx *IGH* FR2 Assay – PGM™
9-121-0047 LymphoTrack® Dx *IGH* FR3 Assay – PGM™
9-122-0009 LymphoTrack® Dx *IGK* Assay Kit A – MiSeq®
9-122-0019 LymphoTrack® Dx *IGK* Assay Panel – MiSeq®
9-122-0007 LymphoTrack® Dx *IGK* Assay – S5/PGM™
9-225-0009 LymphoTrack® Dx *TRB* Assay Kit A - MiSeq®
9-225-0019 LymphoTrack® Dx *TRB* Assay Panel - MiSeq®
9-227-0019 LymphoTrack® Dx *TRG* Assay Kit A – MiSeq®
9-227-0009 LymphoTrack® Dx *TRG* Assay Panel – MiSeq®

## *BCR/ABL* RNA Dilution Sets

4-085-0110 *BCR/ABL* e1a2 RNA Dilution Set
4-085-0210 *BCR/ABL* b3a2 RNA Dilution Set
4-085-0310 *BCR/ABL* b2a2 RNA Dilution Set

## DNA Sensitivity Panels

4-086-0040 Sensitivity Panel – IVS-0004 Clonal Control DNA
4-086-0070 Sensitivity Panel – IVS-0007 Clonal Control DNA
4-086-0090 Sensitivity Panel – IVS-0009 Clonal Control DNA
4-086-0100 Sensitivity Panel – IVS-0010 Clonal Control DNA
4-086-0190 Sensitivity Panel – IVS-0019 Clonal Control DNA
4-086-0210 Sensitivity Panel – IVS-0021 Clonal Control DNA
4-086-0300 Sensitivity Panel – IVS-0030 Clonal Control DNA

## RNA Sensitivity Panels

4-087-0030 Sensitivity Panel – IVS-0003 Clonal Control RNA
4-087-0110 Sensitivity Panel – IVS-0011 Clonal Control RNA
4-087-0150 Sensitivity Panel – IVS-0015 Clonal Control RNA
4-087-0200 Sensitivity Panel – IVS-0020 Clonal Control RNA
4-087-0320 Sensitivity Panel – IVS-0032 Clonal Control RNA

## Cell Line DNA Controls

R-088-0230 *FLT3* ITD Positive Control DNA - GPR
R-088-0240 *FLT3* TKD Positive Control DNA - GPR
R-088-0250 *FLT3* Extraction Control - GPR
4-088-0008 *IGH* SHM Positive Control DNA
4-088-0010 IVS-0001 Clonal Control DNA
4-088-0098 LymphoTrack® B-cell Low Positive Control
4-088-0108 LymphoTrack® T-cell Low Positive Control
4-088-0118 LymphoQuant® B-cell Internal Control
4-088-0128 LymphoQuant® T-cell Internal Control
4-088-0190 IVS-0004 Clonal Control DNA
4-088-0210 20% IVS-0004 Clonal Control DNA
4-088-0220 10% IVS-0004 Clonal Control DNA
4-088-0230 5% IVS-0004 Clonal Control DNA
4-088-0370 IVS-0007 Clonal Control DNA
4-088-0390 20% IVS-0007 Clonal Control DNA
4-088-0400 10% IVS-0007 Clonal Control DNA
4-088-0410 5% IVS-0007 Clonal Control DNA
4-088-0420 1% IVS-0007 Clonal Control DNA
4-088-0430 IVS-0008 Clonal Control DNA
4-088-0470 5% IVS-0008 Clonal Control DNA
4-088-0480 1% IVS-0008 Clonal Control DNA
4-088-0490 IVS-0009 Clonal Control DNA
4-088-0500 30% IVS-0009 Clonal Control DNA
4-088-0510 20% IVS-0009 Clonal Control DNA
4-088-0520 10% IVS-0009 Clonal Control DNA
4-088-0530 5% IVS-0009 Clonal Control DNA
4-088-0540 1% IVS-0009 Clonal Control DNA
4-088-0550 IVS-0010 Clonal Control DNA
4-088-0560 30% IVS-0010 Clonal Control DNA
4-088-0580 10% IVS-0010 Clonal Control DNA
4-088-0590 5% IVS-0010 Clonal Control DNA
4-088-0730 IVS-0013 Clonal Control DNA
4-088-1090 IVS-0019 Clonal Control DNA
4-088-1100 30% IVS-0019 Clonal Control DNA
4-088-1110 20% IVS-0019 Clonal Control DNA
4-088-1120 10% IVS-0019 Clonal Control DNA
4-088-1130 5% IVS-0019 Clonal Control DNA
4-088-1140 1% IVS-0019 Clonal Control DNA
4-088-1210 IVS-0021 Clonal Control DNA
4-088-1220 30% IVS-0021 Clonal Control DNA
4-088-1230 20% IVS-0021 Clonal Control DNA
4-088-1240 10% IVS-0021 Clonal Control DNA
4-088-1250 5% IVS-0021 Clonal Control DNA
4-088-1260 1% IVS-0021 Clonal Control DNA
4-088-1390 IVS-0024 Clonal Control DNA
4-088-1430 5% IVS-0024 Clonal Control DNA
4-088-1690 IVS-0029 Clonal Control DNA
4-088-1700 30% IVS-0029 Clonal Control DNA
4-088-1730 5% IVS-0029 Clonal Control DNA
4-088-1750 IVS-0030 Clonal Control DNA
4-088-1760 30% IVS-0030 Clonal Control DNA
4-088-1770 20% IVS-0030 Clonal Control DNA
4-088-1780 10% IVS-0030 Clonal Control DNA
4-088-1790 5% IVS-0030 Clonal Control DNA
4-088-1800 1% IVS-0030 Clonal Control DNA
4-088-1810 IVS-0031 Clonal Control DNA
4-088-1840 10% IVS-0031 Clonal Control DNA
4-088-1860 1% IVS-0031 Clonal Control DNA

## Cell Line RNA Controls

4-089-0100 IVS-0002 Clonal Control RNA
4-089-0190 IVS-0003 Clonal Control RNA
4-089-0200 $10^{-1}$ IVS-0003 Clonal Control RNA
4-089-0210 $10^{-2}$ IVS-0003 Clonal Control RNA
4-089-0220 $10^{-3}$ IVS-0003 Clonal Control RNA
4-089-0230 $10^{-4}$ IVS-0003 Clonal Control RNA
4-089-0240 $10^{-5}$ IVS-0003 Clonal Control RNA
4-089-0250 $10^{-6}$ IVS-0003 Clonal Control RNA
4-089-0910 IVS-0011 Clonal Control RNA
4-089-0920 $10^{-1}$ IVS-0011 Clonal Control RNA
4-089-0930 $10^{-2}$ IVS-0011 Clonal Control RNA
4-089-0940 $10^{-3}$ IVS-0011 Clonal Control RNA
4-089-0950 $10^{-4}$ IVS-0011 Clonal Control RNA
4-089-0960 $10^{-5}$ IVS-0011 Clonal Control RNA
4-089-1270 IVS-0015 Clonal Control RNA
4-089-1720 IVS-0020 Clonal Control RNA
4-089-1730 $10^{-1}$ IVS-0020 Clonal Control RNA
4-089-1740 $10^{-2}$ IVS-0020 Clonal Control RNA
4-089-1750 $10^{-3}$ IVS-0020 Clonal Control RNA
4-089-1760 $10^{-4}$ IVS-0020 Clonal Control RNA
4-089-2800 IVS-0032 Clonal Control RNA
4-089-2810 $10^{-1}$ IVS-0032 Clonal Control RNA
4-089-2820 $10^{-2}$ IVS-0032 Clonal Control RNA
4-089-2830 $10^{-3}$ IVS-0032 Clonal Control RNA
4-089-2840 $10^{-4}$ IVS-0032 Clonal Control RNA
4-089-2850 $10^{-5}$ IVS-0032 Clonal Control RNA
4-089-2860 $10^{-6}$ IVS-0032 Clonal Control RNA
4-089-3070 IVS-0035 Clonal Control RNA

## Plasmid DNA Control

4-090-0070 IVS-P002 Clonal Control DNA

## Tissue DNA Control

4-092-0010 IVS-0000 Polyclonal Control DNA

## RNA Proficiency Panel

4-310-0100 Proficiency Panel for *BCR/ABL* t(9;22) Translocations

## Somatic Hypermutation Sanger Sequencing Assays

5-101-0030 *IGH* Somatic Hypermutation Assay v2.0 – Gel Detection
5-101-0031 *IGH* Somatic Hypermutation Assay v2.0 – ABI Fluorescence Detection
5-101-0040 *IGH* Somatic Hypermutation Assay v2.0 MegaKit – Gel Detection
5-101-0041 *IGH* Somatic Hypermutation Assay v2.0 MegaKit – ABI Fluorescence

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9-121-0009 LymphoTrack® Dx *IGH* FR1 Assay Kit A – MiSeq®
9-121-0039 LymphoTrack® Dx *IGH* FR1 Assay Panel – MiSeq®

# Alphabetical Index

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## B



## C



## D



## E,F,G,H,I

## A



## B



## C



## D



## E,F,G,H,I

# Notes

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## Patent Notice

Many of the products described herein are covered by one or more of the following: European Patent Number 1549764, European Patent Number 2418287, European Patent Number 2460889, Japanese Patent Number 4708029, United States Patent No. 7,785,783, United States Patent 8859748, United States Patent 10280462, additional United States Patents Pending and planned future applications. All of these patents and applications are licensed exclusively to Invivoscribe®. Additional patents licensed to Invivoscribe covering some of these products apply elsewhere, or as described in this catalog.

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# 2020 Product Catalog

25TH ANNIVERSARY EDITION

invivoscribe®

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