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Product Catalog

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Table of Contents

Introduction

Clonality Overview

Hematological Test Menu

Next Generation Sequencing (NGS)

Capillary (CE-IVD) Assays

Gel and Capillary (RUO) Assays

Analyte Specific Reagents

Controls, Reagents, and Enzymes

Custom Products

Reference

Product List

Improving Lives with Precision Diagnostics®

A technology-driven international company focused on precision diagnostics with best-in-class products and services that optimize patient care and accelerate drug approvals worldwide.

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Dear Colleagues,

As we begin 2023, I want to take a moment to express gratitude to our customers for your hard work and dedication and, further, your feedback on our products that you have provided over the past year. Our mission is to improve patient lives by providing standardized, innovative precision diagnostics. Improving the quality of healthcare worldwide is not easy, and it cannot be achieved alone, so - Thank you!

As in previous years, I am taking this opportunity to highlight a few of our company's many accomplishments in 2022 and identify a few of the many projects we will address in the year ahead.

In May, we announced that we've entered into a strategic partnership with Hitachi High-Tech (HHT). Together, we will advance precision medicine by providing solutions that improve the efficiency and accuracy of cancer diagnosis, prognosis and monitoring throughout the patient's life.

We are pleased to have been selected as a partner for the Foundation for the National Institutes of Health (fNIH) Biomarkers Consortium on a new project to quantify and standardize MRD as a predictor of relapse for patients with acute myeloid leukemia (AML), and we look forward to participating using a combination of both next-generation sequencing (NGS), and multiparametric flow cytometry (MFC) assays. Our NGS assays are standardized and available worldwide; they target both *FLT3* and *NPM1,* the most prevalent and important biomarkers in AML.

In addition, our 4 tube, CAP/CLIA validated AML MRD Assay on the 12-Color BD FACS Lyric has received great reviews testing blind peripheral blood and bone marrow specimens sent by pharma partners down to ~0.005% (LAIP dependent). This MFC assay identifies more than 20 surface markers on AML blasts from background hematopoietic cells with high sensitivity and specificity.

To fulfill an unmet need for *FLT3* MRD, we introduced a next-generation sequencing (NGS)-based RUO *FLT3* ITD MRD Assay, a 24-index kit and Dockerized *FLT3* ITD MRD RUO software, enabling high-throughput laboratories to automate and streamline data analyses. This year we will also release an NGS-based RUO *NPM1* MRD Assay with Dockerized *NPM1* MRD RUO software. We also currently also offer RUO Dockerized LymphoTrack® Enterprise Software compatible with our RUO LymphoTrack clonality assays, enabling large laboratories to establish fully automated data pipelines.

In October, we filed a supplemental Pre-Market Approval (sPMA) submission with the U.S. Food and Drug Administration (FDA) Center for Devices and Radiological Health (CDRH) for the use of the LeukoStrat® CDx *FLT3* Mutation Assay as the companion diagnostic for newly diagnosed AML patients with the *FLT3*-ITD mutation for Daiichi Sankyo's investigational drug quizartinib.

This year we will be aligning our service offerings worldwide, including NGS tests, gene panels, bioinformatics, multiparametric flow cytometry assays and measurable residual disease (MRD) applications to support clinical and pharma partners around the world. To support continued growth in Asia, the Laboratory for Personalized Molecular Medicine (LabPMM) obtained ISO 15189 accreditation in Japan and Invivoscribe Diagnostic Technologies (Shanghai), Co., Ltd. in China obtained CAP accreditation in early 2023. Our China laboratory is our CRO hub for setting up, monitoring, and testing services in China. Central labs in China are expected to have accredited quality management systems to support registrational drug trials. By obtaining CAP accreditation at our China laboratory, Invivoscribe improved its ability to provide drug developers with a premium resource for molecular and flow-based oncology testing.

A new NGS-based service with unprecedented sensitivity, the B-cell MRD Assay, is now available across the LabPMM global network to monitor therapeutic response and serve as a surrogate endpoint in clinical trials.

In our endeavor for continuous improvement, our bioinformatics and R&D teams partnered together to develop an innovative approach of duplexed sequencing and tag-based error correction method in a myeloid gene panel assay to improve sequencing accuracy. Our bioinformatics team also developed SNPrint, a customizable genotyping application developed for our NGS-based gene panels, MyAML®, and MyMRD® to minimize the risk of sample mix-ups.

This year we returned to live conferences, attending multiple conferences across the world where we were able to meet with many of you for the first time in years. We are happy to report that research has commenced in laboratories across the world and our customers generated nearly 50 peer-reviewed scientific publications generated using Invivoscribe products.

The remarkable people at Invivoscribe and LabPMM continue to relentlessly push the limits, working to provide very progressive milestones in the upcoming year:

We expect to be one of the first companies in the world to receive IVDR approval in the EU for our LeukoStrat CDx *FLT3* Mutation Assay and the team is generating IVDR validation packages for multiple capillary and NGS assays for submission to the notified body. Additionally, an NGS-based RUO NPM1 MRD Assay kit will be coming to market —enabling in-house therapeutic monitoring of AML patients.

Our teams are working to introduce a powerful, 11-color multiparametric flow cytometry (MFC) panel, the CLL MRD Assay, which will be provided as a CLIA-validated service throughout our LabPMM global network. The CLL MRD assay will be followed by an array of upcoming MFC-based MRD assays, targeting e.g., B-ALL and Multiple Myeloma.

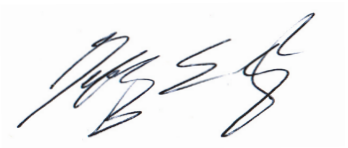
Finally, I want to acknowledge that our growth and accomplishments over nearly 30 years, would not be possible without your feedback and support. We look forward to continued collaboration with research and clinical colleagues to ensure that we can continue to offer you cutting edge products and services for decades to come.

We wish you, your colleagues, and your families a safe, joyful, productive, and successful 2023.

Sincerely Yours,

Jeffrey Edward Miller, Ph.D.

Founder, Chief Scientific Officer,
Chief Executive Officer & Chairman



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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., 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, 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.

Mitchell Kronenberg, Ph.D.

Dr. 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 currently serves as Chief Scientific Officer; he was the President of the institute from 2003 to 2021. 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 400 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, Ph.D.

Dr. Wilson is an immunologist and biotech entrepreneur with more than 30 years of experience in biomedical research, development and executive management. He earned his Ph.D. from the University of Arizona's College of Medicine and joined the La Jolla Institute for Immunology (LJI) in 1997 where he was a fellow of the National Multiple Sclerosis Society and National Institutes of Health. Dr. Wilson entered management at LJI, eventually serving as LJI's Chief Operating Officer. During his tenure the Institute grew substantially, becoming a global leader in immunology, conducting nearly $1B in total research operations and ranking as the #1 place to work in the world by The Scientist magazine. His original research has been published in high impact journals. Dr. Wilson has served as principal or co-principal investigator on more than $75M in competitive grants and awards, including the development of the 2021 X-Prize winning rapid CoVID-19 diagnostic test. Since leaving LJI Dr. Wilson has served in various executive roles in publicly traded companies as President, Chief Executive Officer and Chief Innovations Officer. He is an Associate Clinical Professor at the University of California, San Diego.

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.

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Executive Leadership

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Jeffrey Edward Miller, Ph.D.

Founder, Chief Scientific Officer, and Chief Executive Officer

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.

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, 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.

Tania Obranovich, BSc (Hons) PhD LLB

Chief IP and Legal Strategy Officer

Dr. Obranovich has more than 25 years' experience developing and managing global IP portfolios in the biotechnology sector, in particular in relation t o molecular, cellular and genomic technologies as they relate to diagnostics and therapeutics. She has acquired this experience both through private practice, having spent more than a decade as a partner in a top tier IP firm, and as legal counsel for a US based diagnostics company where she ran patent and trade mark prosecution and drafting, in addition to overseeing all legal, compliance and corporate governance matters. Tania has practiced throughout her career as both a lawyer and patent attorney. Prior to these roles she completed a PhD and post-doctoral fellowship in immunology. In addition to her legal practice, Tania has lectured extensively in patent law at The University of Melbourne and Monash University and also speaks widely in relation to IP issues. She was heavily involved with navigating the Australian and US legal and policy issues that arose as a result of the Myriad gene patent litigation in both these jurisdictions. Tania received a Federal Government appointment to the Professional Standards Board for Patent and TM Attorneys in 2012 and has held several other Board positions. Tania has served as Chief IP and Legal Strategy Officer to Invivoscribe since 2019.

Dr. Meindert Niemeijer, Ph.D., M.Sc.

Chief Information Officer

Dr. Niemeijer came to IVS from Digital Diagnostics where he led the teams that designed, built, documented and put into production the first autonomous diagnostic Artificial Intelligence (AI), cleared by the FDA. He is an experienced technology leader and deep learning expert passionate about developing AI based medical devices. During his career, he has built a deep expertise in medical device (software) development processes and the way in which these interact with quality management and other regulatory requirements. He loves building and developing technology, processes and teams. Dr. Niemeijer obtained a Ph.D. in Medical Image Analysis and Machine Learning from Utrecht University in the Netherlands. In addition, he has an M.Sc. in Medical Computer Science, also from Utrecht University.

Tony Lialin

Chief Commercial Officer

Tony Lialin joined Invivoscribe in 2021 as Chief Commercial Officer. In this role he is responsible for leading the global commercial organization. Mr. Lialin has more than two decades of experience in growing companies in the life sciences industry having held positions in clinical diagnostic, bioinformatics software, instrumentation and reagent companies. He has successfully scaled commercial operations at three start-ups resulting in those companies being purchased by large public companies. Mr. Lialin comes to IVS from Loop Genomics, an NGS long-read sequencing company where he started their global commercial operations growing their business to several million dollars in revenue per year in just three years. From 2014 through June 2018, Mr. Lialin managed $400M in portfolio business at Illumina working closely with customers in the DTC, Oncology and Postnatal testing spaces. He also served as a director at Agilent Technologies, where he managed the emerging market, field application scientist, and technical support and field service engineering teams. As a six-sigma green belt he values process, service as a differentiator and both internal and external customers. Mr. Lialin earned a BA in Molecular Cellular and Developmental Biology from the University of California at Santa Cruz.

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Leadership Team

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Andreia de Albuquerque, Ph.D.

Director of Global Business Development

Dr. de Albuquerque joined Invivoscribe in 2015 and currently serves as the Director of Business Development. In this role, she is responsible for leading Invivoscribe's global partnerships with key Pharma and Biotech companies, focusing on companion diagnostics development, custom assay development, clinical trial testing, corporate partnerships and other strategic relationships. Prior to joining Invivoscribe, Dr. de Albuquerque held roles in precision molecular diagnostics, focusing on development, implementation and validation of PCR and NGS assays for diagnostic and research purposes. Her work has resulted in several peer-reviewed publications and participations in international conferences. During her career, she has at times been responsible for overseeing laboratory diagnostic teams and operations. Dr. de Albuquerque's extensive expertise in precision diagnostics and understanding of assay development and clinical testing, paved the way into Business Development, where she now focuses on accelerating drug approvals. Her approach is to build successful partnerships, provide technical expertise and work closely with biomarker leaders, lab operations, clinical scientists and regulatory groups.

Dr. de Albuquerque earned her degree in Biology from the University of Coimbra (Portugal) and a combined Ph.D. in Oncology and Molecular Biology from the University of Dresden (Germany).

Paul McMullin, MIM, MBA

Head of Global Sales and 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.

Jordan Thornes

Global Director, Clinical Labs

Jordan Thornes joined Invivoscribe in 2004 and currently serves as the Global Director of Clinical Labs. In this role, he is responsible for leading Invivoscribe's global network of clinical labs, The Laboratory for Personalized Molecular Medicine®, (LabPMM®). There are currently four such LabPMM facilities which are strategically located globally in San Diego, USA; Hallbergmoos (Munich), Germany; Shanghai, China and Kawasaki (Tokyo), Japan. LabPMM aids patients and pharmaceutical companies worldwide with globally standardized and clinically-actionable diagnostic methods. LabPMM utilizes proprietary biomarkers to select the correct patients and monitor and track their critically important response throughout the course of their disease.

Ying Huang, Ph.D.

Senior Director of Product Development

Dr. Huang joined Invivoscribe in 2010 and currently serves as the Sr. Director of Product Development. In this role, she is responsible for leading Invivoscribe's product development activities to address unmet clinical needs in the fields of molecular diagnostic and precision medicine. Dr. Huang has over 25 years of research and industry experience with a broad knowledge in technology and assay development. She is passionate about developing novel methods using cutting edge technologies to improve lives with precision diagnostics. She has successfully led development teams through entire product development life cycle, from feasibility to RUO, CLIA and IVD grade product launches and post market support under respective regulatory standards. Prior to Invivoscribe, Dr. Huang worked at Illumina, ACEA Biosciences, Nanogen and MD Anderson Cancer Center. In those roles, she developed a variety of techniques ranging from isolating CTC using microfluidic devices to detecting biowarfare agents using electronic microarray. Dr. Huang was the PI for research grants from DARPA and NIH. She is the co-author of more than 60 publications and meeting abstracts and is the co-inventor of 9 patents. Dr. Huang received her Ph.D. in Electrical Engineering from Bangor University, UK and B.E. in Electronic Engineering from Xidian University, China.

Jason Gerhold

Global Director, Regulatory Affairs/Quality Assurance

Jason Gerhold joined Invivoscribe in 2012 and serves as the Global Director of Regulatory, Quality, and Clinical Affairs focusing on developing high quality diagnostics that meet both international pathology needs and support pharma partner's drug development programs. He directly manages employees in the US, Japan, and China, and is responsible for worldwide compliance to relevant laws, regulations, and standards. Mr. Gerhold has over 20 years of experience in the biotech industry with biologic therapeutics and diagnostics, including process development, regulatory, quality, and clinical roles. Mr. Gerhold has worked in small, medium and large companies; successfully registering IVD and CDx products in several international markets, developing, improving, and maintaining quality management systems while serving as the management representative to regulatory authorities, and leading clinical teams managing international registrational studies. He is known for building strong rapport with regulators and working well with research and pharma teams resulting in highly successful partnerships. He has completed trainings and/or accreditations for ISO 13485 Lead Auditor, Six Sigma Greenbelt, Clinical Trials Administration, Project Management, and CTD writing.

Best-in-Class Assays and 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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Next-Generation Sequencing (NGS)

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. Our NGS assays provide comprehensive solutions from diagnosis through Measurable Residual Disease tracking.

ABI Fluorescence Detection and Gel Detection

We exclusively offer a comprehensive selection of PCR-based assays for ABI fluorescence detection and 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 and Reagents

Invivoscribe® offers an extensive range of General Purpose Reagents (GPRs) and Research Use Only (RUO) reagents. Our high quality, reliable DNA and RNA controls, master mixes, and enzymes, are all manufactured under cGMP conditions to guarantee reliability.

Companion Diagnostics (CDx)

Streamline drug approvals leveraging our Companion Diagnostic (CDx) assay development, manufacturing and regulatory expertise to accelerate drug approvals. Custom assays are designed and manufactured under an ISO certified quality management system.

LabPMM® Clinical Lab Services

Invivoscribe's international network of clinical labs, Laboratory for Personalized Molecular Medicine® (LabPMM®), provides globally-standardized testing of novel and proprietary biomarkers that are critical for patient care.

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invivoscribe.com/clinical-lab-services/

NGS Cancer Panels

Identification of chromosome abnormalities and actionable driver mutations aid in the stratification of patient risk, and provide critical insight for patients with leukemia or lymphoma.

NGS MRD Tests

Track disease and trend treatment response by monitoring clonal B- (Ig) and T-cell (TCR) gene rearrangements as well as *FLT3* ITD and *NPM1* mutations with unprecedented sensitivity.

Clonality Tests

Malignant lymphoproliferative disorders collectively represent a large portion of hematological cancers and are characterized by reduced population diversity of Ig and TCR rearrangements. Confirm diagnostic suspicions and identify hematological neoplasms with LabPMM's NGS Clonality services, available for Ig, TCR, and somatic hypermutation (SHM).

Companion Diagnostic (CDx) Tests

CDx enable the identification of biomarkers that indicate the patients most likely to benefit (or alternately not benefit) from specific or combinatorial targeted therapies. Their utilization enhances patient care and the potential for improved outcomes. 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] The LeukoStrat CDx *FLT3* Mutation Assay is a globally standardized test validated for the detection of internal tandem duplications (ITD) and tyrosine kinase domain (TKD) mutations of the *FLT3* gene.

Custom Assays

Invivoscribe's experienced regulatory team has over 50 product registrations and multiple companion diagnostic approvals, allowing for optimal strategy, streamlined development and milestone achievement. With an ever-changing regulatory landscape, why take a chance with anyone else?

Targeted Genes

FLT3 and *NPM1* are offered to detect targeted mutations. *FLT3* and *NPM1* mutations can be used for risk stratification and prognosis of AML.

Multiparametric Flow Cytometry

Complement NGS testing with globally-standardized, 12-color flow cytometry testing. Our comprehensive assay designs allow greater specificity and sensitivity using smaller sample volumes as compared to 4- or 6-fluorochrome tests. Some of the many MFC applications include leukemia and lymphoma diagnosis and screening and rare event analyses such as MRD.

References
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 Partnerships

Companion Diagnostics represents a critical milestone in precision medicine and plays a pivotal role in the validation of targeted therapies. Use of a single partner for CDx development minimizes complexity and risk which could affect drug approvals. Our Streamlined CDx™ program supports all stages of development, validation, regulatory submission, and commercialization. Let us help you accelerate your drug approval.

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Product Development

Design Controls: All biomarker assays & software developed under design controls.

Bioinformatics Software: Comprehensive solutions to prevent errors, streamline workflow and reduce turn-around times.

Identify and Track: NGS panels and targeted assays to identify clonal architecture and track disease burden.

Custom Development: Custom biomarker assay development.

Complementary MRD Assays: MRD assays may be used as surrogate endpoints for P3 clinical trials.

Manufacturing

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

CE-marked IVDs and CDx Manufacturing: CE-Marked, PMDA and FDA approved CDx, and >50 CE-IVDs including gel, capillary electrophoresis and NGS assays.

Assay Development: IUO & RUO assays, CE-marked IVDs, & CDx.

Controls and Reagents: DNA / RNA controls, ASRs, GPRs, MRD controls & proficiency panels.

Clinical Lab Services

Clinical Lab Experience: More than a dozen years of clinical reference lab experience.

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

Comprehensive Panels and Targeted Assays: Comprehensive NGS and MFC-based panels to screen, identify and track clinically actionable biomarkers.

MOA Profiling of Novel Compounds: Using primary AML patient samples - IC50 determination, mRNA analysis, genetic, and phenotypic characterization.

Worldwide Enrollment: Testing services have supported hundreds of enrollment sites worldwide.

Global Regulatory Expertise

Accredited and 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 companion diagnostics that are CE-marked, FDA and PMDA/MHLW approved.

CE-marked IVDs: More than 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.

Global Distribution 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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Global Invivoscribe® Locations

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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Background

PCR and NGS-Based Assessment of Clonality in Hematologic Malignancies

For nearly 30 years, 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 measurable 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 more 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

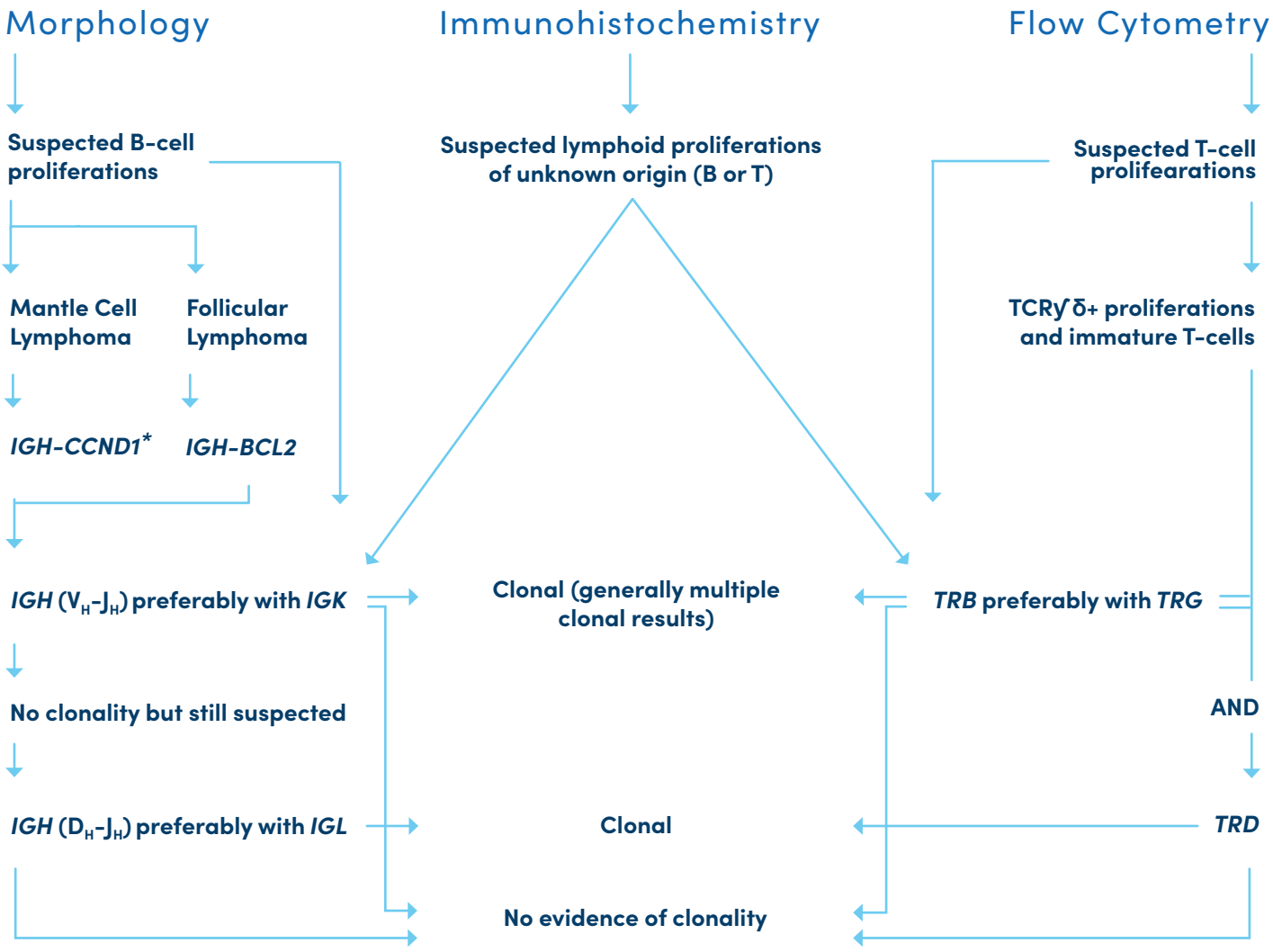
The adaptive vertebrate immune system produces a repertoire of immunoglobulin and T-cell receptor molecules using a relatively limited number of heritable germline gene segments. Somatic gene rearrangement is the fundamental mechanism used to generate different immunoglobulin and T-cell receptor molecules, 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]

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

If a definitive diagnosis is not possible following:



EuroClonality

*Previously known as *BCL1/*J_H. Results should be considered in the context of all available clinical, histological and immunophenotypic data.

Hematological Test Menu

References

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	Diseases	Gene Rearrangement								Translocation				Mutation	
		IGH V_H - J_H	*IGH* D_H - J_H	*IGK*	*IGL*	*IGHV* SHM	*TRB*	*TRD*	*TRG*	*IGH-BCL1* (CCND1)	*IGH-BCL2*	*BCR-ABL1**	*PML RARα**	*FLT3*	*NPM1*
Lymphoid/ Lymphoma	Marginal Zone Lymphoma (MZL), extranodal[12,13,27]	88%	58%	84%	29%		23%	10%	16%						
	Marginal Zone Lymphoma (MZL), nodal[13]	100%	30%	80%	30%		10%	20%	10%						
	Mantle Cell Lymphoma (MCL)[2,6,7,12,13,27,37]	100%	11%	100%	44%	*	9%	4%	11%	75%					
	Follicular Lymphoma (FL)[3,7,12,13,27,28]	84%	19%	84%	21%		6%	5%	2%		90%				
	Diffuse Large B-cell Lymphoma (DLBCL)[3,12,13,27]	80%	30%	80%	28%		21%	14%	15%		30%				
	Multiple Myeloma (MM) and other Plasma Cell Neoplasms (PCN)[2,9,10,20,25]	84%	60%	57%	97%					20%					
	Chronic Lymphocytic Leukemia (CLL)[11,12,13,15,23,27,35]	100%	43%	100%	30%	*	25%	12%	18%						
	B-cell Acute Lymphoblastic Leukemia (B-ALL)[4,12,14,19,21,22,27,29,30,31,32,33,34]	96%	57%	95%	20%		81%	86%	75%			30%			
	Suspect B-cell Proliferations[12,26,27,33]	93%	93%	90%	40%		20%		20%						
	Peripheral T-cell Lymphoma (PTCL)[12,13,14,24]	35%	4%		2%		98%		94%						
	T-cell Acute Lymphoblastic Leukemia (T-ALL)[12,14,21,22,29,31]	24%	25%	4%			92%	68%	95%						
	Angioimmunoblastic T-cell Lymphoma (AILT)[12,13,14]	19%	11%	30%	5%		99%	35%	92%						
	Adult T-cell Leukemia/Lymphoma[39]						97%		96%						
	Anaplastic Large-Cell Lymphoma (ALCL)[12,13,14]						74%	12%	74%						
	T-cell Prolymphocytic Leukemia (T-PLL)[12,13,14]	3%	3%	3%	3%		100%	6%	94%						
	T-cell Large Granular Lymphocytic Leukemia (T-LGL Leukemia)[12,13,14]			4%	4%		97%	29%	96%						
	Suspect T-Cell Proliferations[12,26,40]	10%		10%			90%	11%	90%						
Myeloid	Acute Myeloid Leukemia (AML)[6,16]													33%	64%
	Acute Promyelocytic Leukemia (APL)[1,5,16,17]												90%		
	Chronic Myeloid Leukemia (CML)[7,18,19,21,38]											87%			
	Myeloproliferative Neoplasms (MPNs)[38]											10%			

Note: The percentage of samples within a given disease category were detected using each gene target. Percentages indicate the highest referenced value.

*Some translocation assays, LymphoTrack®, and MRD applications are currently Research Use Only; not for use in diagnostic procedures. Illumina® and MiSeq™ are registered trademarks of Illumina®, Inc. Ion S5™ and Ion PGM™ are trademarks of Thermo Fisher Scientific®.

Recommended Primary Test

Recommended Secondary Test

LymphoTrack® Dx

Next-Generation Squencing (NGS) CE-IVD Assays

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

LEARN MORE:
catalog.invivoscribe.com

Introduction

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 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 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.

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
- Bioinformatics software is available in desktop or Docker images for analysis and interpretation
- Same reagents for clonality, somatic hypermutation (SHM), minimal residual disease (MRD) testing, and tracking/monitoring of immunotherapy constructs

Chapter Contents

NGS Assays

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* 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 (Catalog #: 91210007).

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 master mixes target the Leader (V_HL) and the joining (J_H) 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 and pooling of amplicons from several different samples and targets into a single Illumina® MiSeq™ run. No post-PCR ligation step is required.

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 (V_H) gene segments, 27 functional diversity (D_H) gene segments, and 6 functional joining (J_H) 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 V_H-D_H-J_H rearrangements that are unique in both length and sequence.[1]

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 V_HL 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)[2] and small lymphocytic lymphoma (SLL). In addition, NGS methods can improve disease stratification.

Specimen Requirement

50 ng of high-quality genomic DNA.

References

1. Miller et al., *Molecular Genetic Pathology* (2nd ed.). Springer Science & Business Media. 2013: 302.2.7.13 and 30.2.7.18.
2. Ghia et al., *Leukemia* 21: 2-3 (2007).

Simplified representation of the immunoglobulin heavy chain (*IGH*) gene locus on chromosome 14. Depicted are the variable (V_H) and downstream consensus joining (J_H) region genes involved in rearrangements. Upstream of the variable gene segments, the leader sequence (V_HL) 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. 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 Software Package

CE IVD These products are CE-IVD assays for *in vitro* diagnostic use and available in regions that accept CE-IVD products.

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 and gene segment utilization.
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.

These assays utilize a single multiplex master mix to target each conserved *IGH* Framework Region (FR1, FR2, and FR3) within the V_H and the J_H regions described in lymphoid malignancies. Each master mix targets one of the conserved *IGH* framework regions (FR1, FR2, or FR3) within the V_H and the J_H regions described in lymphoid malignancies. Targeting all three framework regions significantly reduces the risk of not being able to detect the presence of clonality, as somatic hypermutations in the primer binding sites of the involved V_H gene segments can impede DNA amplification.[1] 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. Positive clonal (SHM negative) and negative polyclonal DNA controls are included in kits. A clonal SHM positive control can be purchased separately (cat#: 40880008).

Background

The human immunoglobulin heavy chain (*IGH*) gene locus on chromosome 14 (14q32.3) includes 46-52 functional and 30 non-functional variable (V_H), 27 functional diversity (D_H), and 6 functional joining (J_H) 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.[2] Specifically during B-cell development, *IGH* molecules are assembled from multiple polymorphic gene segments that undergo rearrangements generating V_H-D_H-J_H combinations unique in both length and sequence.[3] 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.[4]

*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.

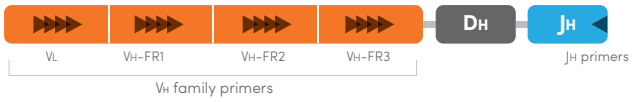
Specimen Requirement

50 ng of high-quality genomic DNA.

References

1. Evans, P. A. et al., (2007). *Leukemia* 21, 207-14.
2. Tonegawa, S. (1983). *Nature* 302, 575-581.
3. Miller JE. (2013) *Molecular Genetic Pathology* (2nd Edition.,sections 30.2.7.13 and 30.2.7.18).
4. Ghia et al., *Blood* 105:1678-1685 (2005).

Simplified Representation of the *IGH* Gene



Simplified depiction of variable (V_H) and downstream consensus joining (J_H) 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

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

LymphoTrack Dx *IGH* FR2 Components

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

LymphoTrack Dx *IGH* FR3 Components

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

*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.

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

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

LymphoTrack Dx *IGH* FR2 Components

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

LymphoTrack Dx *IGH* FR3 Component

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

Controls in Individual FR (1,2, or 3) Kits

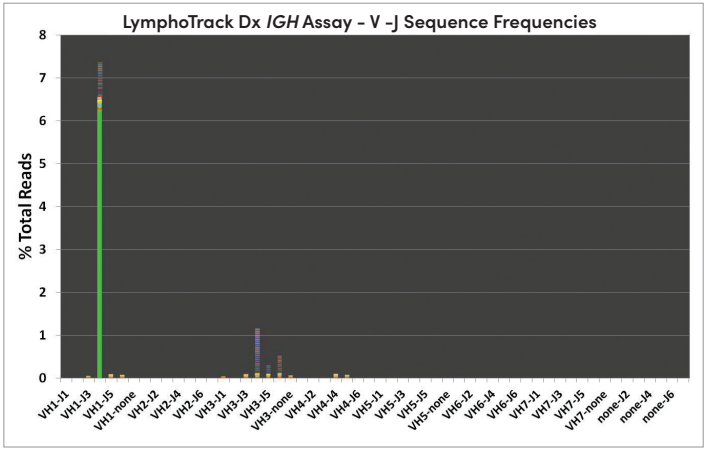
IGH POS (+) Qty. 2

NGS NEG (-) Qty. 2

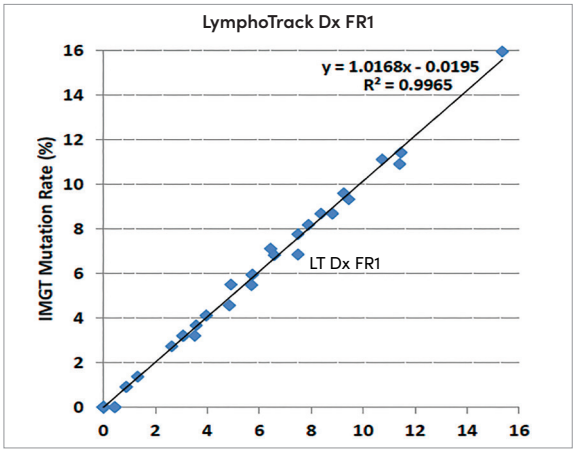
Controls in FR 1/2/3 Kit

IGH POS (+) Qty. 4

NGS NEG (-) Qty. 4



V-J Sequence Frequencies. The LymphoTrack Dx Software provides a stacked bar graph depicting the relative frequencies of the 200 most prevalent V_H-J_H 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 for Reagents – Miseq™ Detection

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 Software Package

Ordering Information for Reagents – Ion S5™ / PGM™ Detection

Catalog #	Products	Quantity Components
9-121-0057	LymphoTrack® Dx I*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 Software Package

CE IVD These products are CE-IVD assays for *in vitro* diagnostic use and available in regions that accept CE-IVD products.

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.

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

LymphoTrack Dx Software is capable of sorting complex NGS data generated by LymphoTrack Dx Assays 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.

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 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 LymphoTrack MRD Software.

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.[1] 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.[2]

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. Tonegawa, S. (1983). *Nature* 302, 575-581.
2. Miller JE. (2013) *Molecular Genetic Pathology* (2nd Edition.,sections 30.2.7.13 and 30.2.7.18).

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}).

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

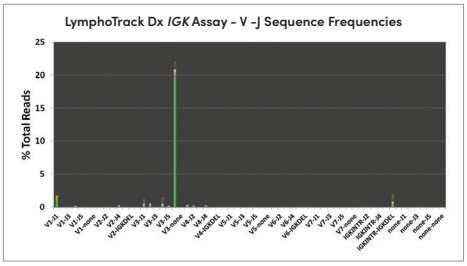
Assay Components

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 Software Package
9-122-0007	LymphoTrack® Dx *IGK* Assay - S5/PGM™	12 indices - 5 sequencing reactions each
9-500-0007	LymphoTrack® Dx Software - S5/PGM™	1 Software Package

CE IVD These products are CE-IVD assays for *in vitro* diagnostic use and available in regions that accept CE-IVD products.

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

This assay utilizes a single multiplex master mix to target conserved V and J regions of the human *TRG* gene locus described in lymphoid malignancies, thereby reducing sample DNA requirements and simplifying the testing workflow. 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.

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

LymphoTrack Dx Software sorts complex NGS data generated by LymphoTrack Dx Assays 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.

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.[1] 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.[2] 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.

Note: For a more thorough explanation of the locus and the targeted deep sequencing strategy, please refer to Principle of Immunoglobulin and T-Cell Receptor Gene Rearrangement.[2]

Specimen Requirement

50 ng of high-quality genomic DNA.

References

1. Tonegawa, S. (1983). *Nature* 302, 575-581.
2. Miller JE. (2013) *Molecular Genetic Pathology* (2nd Edition.,sections 30.2.7.13 and 30.2.7.18).

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.

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

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. 1

NGS NEG (-) Qty. 1

Controls

TRG POS (+) Qty. 3

NGS NEG (-) Qty. 3

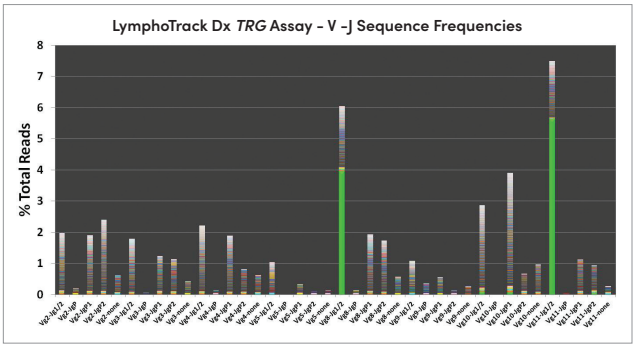
Reagents - Ion S5™/PGM™ Detection

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 Software Package
9-227-0007	LymphoTrack® Dx *TRG* Assay - S5/PGM™	12 indices - 5 sequencing reactions each
9-500-0007	LymphoTrack® Dx Software - S5/PGM™	1 Software Package

CE IVD These products are CE-IVD assays for *in vitro* diagnostic use and available in regions that accept CE-IVD products.

LymphoTrack® Dx *TRB* Assay

Assay Description

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

Summary and Explanation of the Test

This assay utilizes a single multiplex master mix to target conserved V and J regions of the human *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.

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

LymphoTrack Dx Software is capable of sorting complex NGS data generated by LymphoTrack Dx Assays - MiSeq™ 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 Assay from the MiSeq instrument (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.

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.[1] 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.[2]

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.

Note: For a more thorough explanation of the locus and the targeted deep sequencing strategy, please refer to Principle of Immunoglobulin and T-Cell Receptor Gene Rearrangement.[2]

Specimen Requirement

50 ng of high-quality genomic DNA.

References

1. Tonegawa, S. (1983). *Nature* 302, 575-581.
2. Miller JE. (2013) *Molecular Genetic Pathology* (2nd Edition.,sections 30.2.7.13 and 30.2.7.18).
3. JE Miller et al., *Molecular Genetic Pathology* (2nd ed.). Springer Science & Business Media. 2013: 30.2.7.13.

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

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

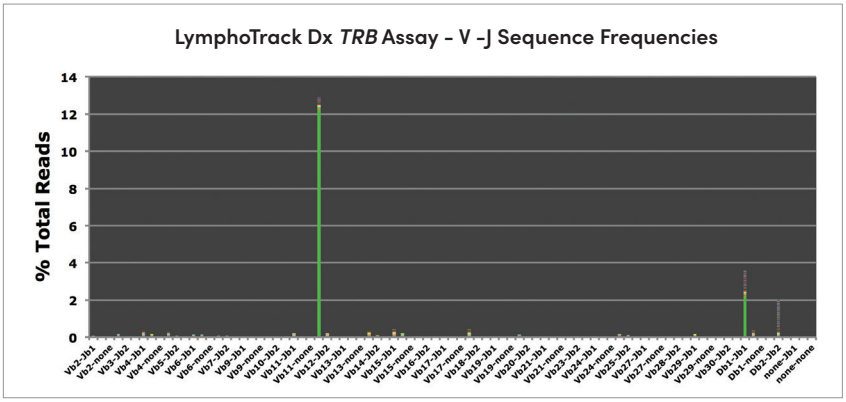
TRB POS (+) Qty. 1

NGS NEG (-) Qty. 1

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 Software Package

CE IVD These products are CE-IVD assays for *in vitro* diagnostic use and available in regions that accept CE-IVD products.

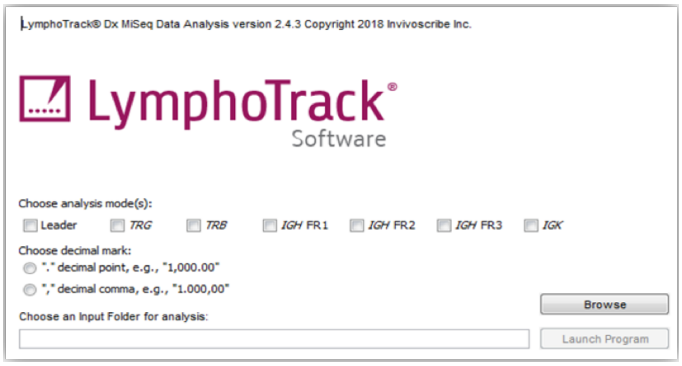
LymphoTrack® Dx Bioinformatics Software

Software Use

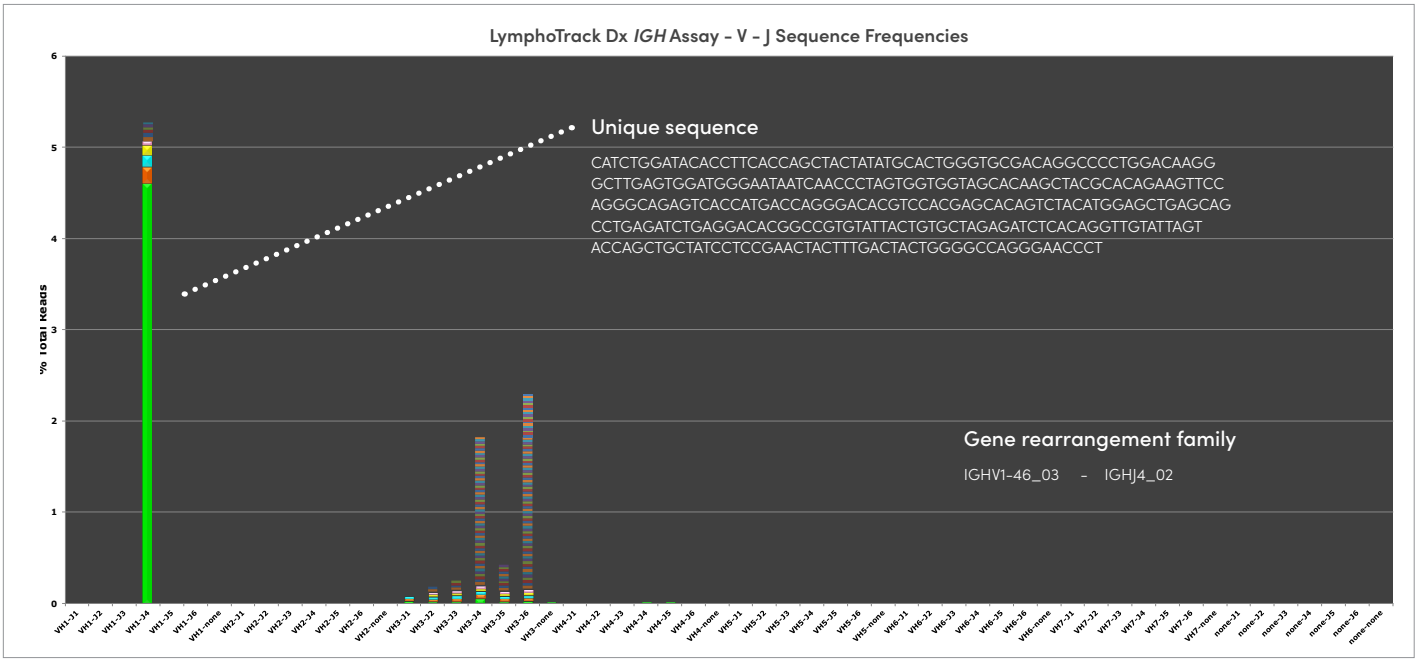
The LymphoTrack Dx Bioinformatics Software package is designed to analyze raw FASTQ files generated by LymphoTrack Dx Assays for clonality analysis of single or multiple target data sets (*IGHV* Leader, *IGH* FR1, *IGH* FR2, *IGH* FR3, *IGK, TRB*, or *TRG*). For data generated with LymphoTrack Dx *IGHV* 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 LymphoTrack Dx 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.



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 LymphoTrack Dx Assay instructions for use (IFU), interpretation is quick and easy.

1 Sample Name

Total reads = 32,458

Rank	2 Sequence	Length	Merge count	3 V-gene	J-gene	4 % Total reads	Cumulative %	5 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 bp 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. The software for LymphoTrack Dx *IGHV* Leader and *IGH* FR1 Assays automatically calculates the somatic hypermutation status of a sequence 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 Software Package
9-500-0007	LymphoTrack® Dx Software - S5/PGM™	1 Software Package

CE IVD These products are CE-IVD assays for *in vitro* diagnostic use and available in regions that accept CE-IVD products.

LymphoTrack®

Next-Generation Squencing (NGS) Research Use Only (RUO) Assays

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

LEARN MORE:
catalog.invivoscribe.com

Introduction

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 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.

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
- Bioinformatics software for analysis and interpretation
- Same reagents for clonality, somatic hypermutation (SHM), measurable residual disease (MRD) testing, and tracking/monitoring of immunotherapy constructs

Chapter Contents

NGS 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 Assays

Assay Uses

This research use only (RUO) assay for next-generation sequencing (NGS), identifies clonal *IGH* V_H-J_H rearrangements, the associated V_H-J_H DNA sequences, and the frequency distribution of V_H region and J_H 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 (71210007).

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 (V_HL) and the joining (J_H) 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.

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

LymphoTrack Software is designed to sort complex NGS data generated from LymphoTrack Assays by gene target, providing easy bioinformatics analyses and data visualization. Once the clonal sequence(s) are identified, they can be monitored/tracked using the LymphoTrack MRD Software 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 (V_H) gene segments, 27 functional diversity (D_H) gene segments, and 6 functional joining (J_H) 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 V_H-D_H-J_H combinations that are unique in both length and sequence.[1]

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) [2] 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. Miller et al., *Molecular Genetic Pathology* (2nd ed.). Springer Science & Business Media. 2013: 302.2.7.13 and 30.2.7.18.
2. Ghia et al., *Leukemia* 21: 2-3 (2007).

Simple representation of the organization of the immunoglobulin heavy chain (*IGH*) gene on chromosome 14. Depicted are the variable region (V_H) genes and downstream consensus joining region genes (J_H) that are involved in rearrangements. Upstream of the variable gene segments, the leader sequence (V_HL) 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 Software Package

These products are for Research Use Only (RUO). Not intended for diagnostic purposes.

LymphoTrack® *IGH* FR1/FR2/FR3 Assays

Assay Uses

These research use only (RUO) assays for next-generation sequencing (NGS), identify clonal *IGH* V_H–J_H rearrangements, the associated V_H–J_H region DNA sequences, the frequency distribution of V_H region and J_H 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 V_H–J_H 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.

Each single multiplex master mix targets one of the conserved *IGH* framework regions (FR1, FR2, or FR3) within the V_H and the J_H regions described in lymphoid malignancies. **Targeting all three framework regions significantly reduces the risk of not being able to detect the presence of clonality**, as somatic hypermutations in the primer binding sites of the involved V_H gene segments can impede DNA amplification.[1]

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.

Positive clonal (SHM negative) and negative polyclonal DNA controls are included in kits. A clonal SHM positive control can be purchased separately (Catalog # 40880008).

LymphoTrack Software is designed to sort complex NGS data generated from LymphoTrack Assays by gene target, providing easy bioinformatics analyses and data visualization. Once the clonal sequence(s) are identified, they can be monitored/tracked using the LymphoTrack MRD Software 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 (V_H) gene segments, 27 functional diversity (D_H) gene segments, and 6 functional joining (J_H) gene segments spread over 1250 kilobases.

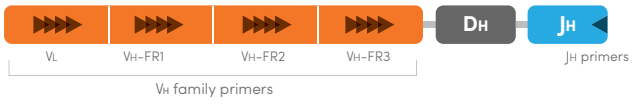
During development of lymphoid cells, the antigen receptor genes go through somatic gene rearrangements.[1] For example, during B-cell development, genes encoding the *IGH* molecules are assembled from multiple polymorphic gene segments that undergo rearrangements and selection, generating V_H-D_H-J_H 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

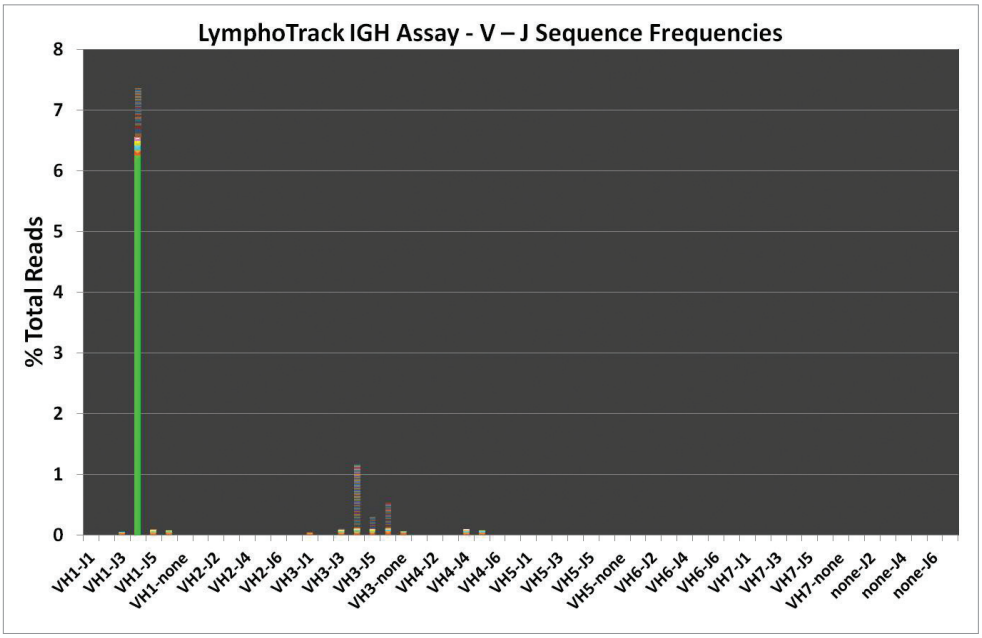
50 ng of high-quality genomic DNA.

Reference

1. S Tonegawa. *Nature* 302: 575–581 (1983).



Simple representation of the organization of the immunoglobulin heavy chain (*IGH*) gene locus on chromosome 14. Depicted are the variable region (V_H) genes and downstream consensus joining region genes segments (J_H) 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 V_H–J_H 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™	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 Software Package
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 Software Package
7-500-0008	LymphoTrack® MRD Software	1 Software Package

These products are for Research Use Only (RUO). Not intended for diagnostic purposes.

Reagents - MiSeq™ Detection

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

LymphoTrack *IGH* FR1 Components

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

LymphoTrack *IGH* FR2 Components

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

LymphoTrack *IGH* FR3 Components

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

Reagents - MiSeq™ Detection Continued

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

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 38	A038				
IGH FR1 MiSeq 39	A039				
IGH FR1 MiSeq 40	A040				
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

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

These products are for Research Use Only (RUO). Not intended for diagnostic purposes.

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.

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

LymphoTrack Software is designed to sort complex NGS data generated from LymphoTrack Assays by gene target, providing easy bioinformatics analyses and data visualization. Once the clonal sequence(s) are identified, they can be monitored/tracked using the LymphoTrack MRD Software 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 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.[1] 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.[2] 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.

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

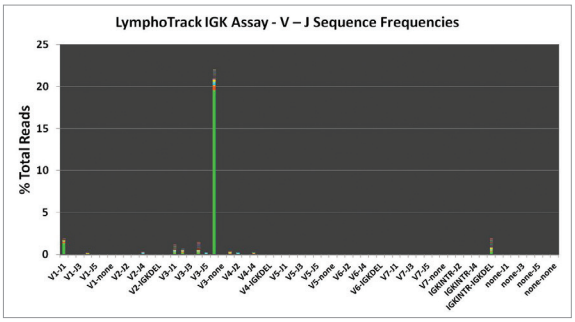
Assay Components

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 Software Package
7-122-0007	LymphoTrack® *IGK* Assay - S5/PGM™	12 indices - 5 sequencing reactions each
7-500-0007	LymphoTrack® Software - S5/PGM™	1 Software Package
7-500-0008	LymphoTrack® MRD Software*	1 Software Package

*MRD Software can be used to track sequences generated by either LymphoTrack Assays - MiSeq™ or Ion S5/PGM™.
These products are for Research Use Only (RUO). Not intended for diagnostic purposes.

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.

This assay utilizes a single multiplex master mix to target conserved V and J regions of the human *TRG* gene locus described in lymphoid malignancies, thereby reducing sample DNA requirements and simplifying the testing workflow. 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.

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

LymphoTrack Software is designed to sort complex NGS data generated from LymphoTrack Assays by gene target, providing easy bioinformatics analyses and data visualization. Once the clonal sequence(s) are identified, they can be monitored/tracked using the LymphoTrack MRD Software 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 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.[1] These developmentally regulated gene rearrangements generate V-J combinations that are unique for each cell.[2] 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.

Note | For a more thorough explanation of the locus and the targeted deep sequencing strategy, please refer to Principle of Immunoglobulin and T-Cell Receptor Gene Rearrangement.[2]

Specimen Requirement

50 ng of high-quality genomic DNA.

References

1. Tonegawa, S. (1983). Nature 302, 575-581.
2. Miller JE. (2013) Molecular Genetic Pathology (2nd Edition.,sections 30.2.7.13 and 30.2.7.18).

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

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. 1 NGS NEG (-) Qty. 1

Controls

TRG POS (+) Qty. 3 NGS NEG (-) Qty. 3

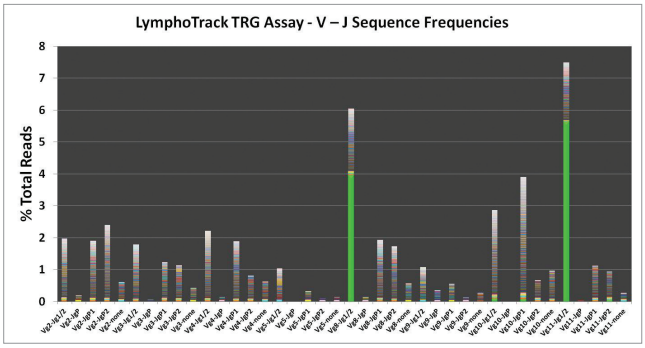
Reagents - Ion S5/PGM™ Detection

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 Software Package
7-227-0007	LymphoTrack® *TRG* Assay - S5/PGM™	12 indices - 5 sequencing reactions each
7-500-0007	LymphoTrack® Software - S5/PGM™	1 Software Package
7-500-0008	LymphoTrack® MRD Software*	1 Software Package

*MRD Software can be used to track sequences generated by either LymphoTrack Assays - MiSeq™ or Ion S5/PGM™.
These products are for Research Use Only (RUO). Not intended for diagnostic purposes.

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, and provides the frequency distribution of Vβ, Dβ, and Jβ region segment utilization using the Illumina® MiSeq™ platform.

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.

Summary and Explanation of the Test

This assay utilizes a single multiplex master mix to target conserved V, D and J regions of the human *TRB* gene locus described in lymphoid malignancies, thereby reducing sample DNA requirements and simplifying 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.

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

LymphoTrack Software is designed to sort complex NGS data generated from LymphoTrack Assays by gene target, providing easy bioinformatics analyses and data visualization. Once the clonal sequence(s) are identified, they can be monitored/tracked using the LymphoTrack MRD Software 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 LymphoTrack *TRB* Assay represent a significant improvement over clonality assessment by fragment analysis by providing two 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 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.[1] 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.[2]

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.

Note: For a more thorough explanation of the locus and the targeted deep sequencing strategy, please refer to Principle of Immunoglobulin and T-Cell Receptor Gene Rearrangement.[2]

Specimen Requirement

50 ng of high-quality genomic DNA.

References

1. Tonegawa, S. (1983). Nature 302, 575-581.
2. Miller JE. (2013) Molecular Genetic Pathology (2nd Edition.,sections 30.2.7.13 and 30.2.7.18).

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

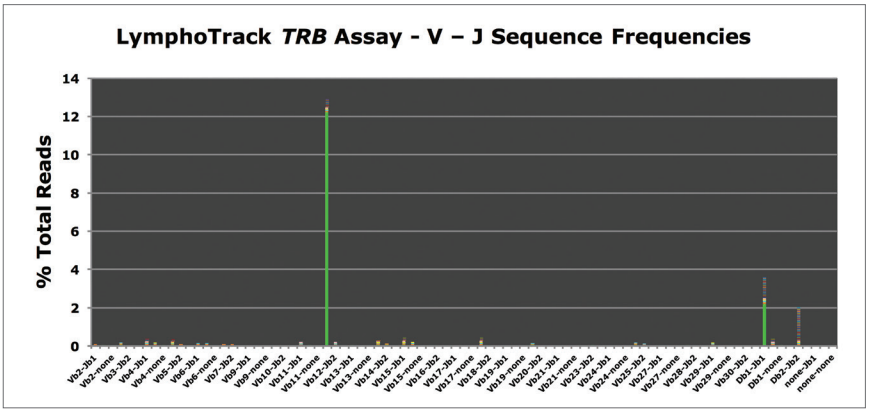
TRB POS (+) Qty. 1	NGS NEG (-) Qty. 1

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 Software Package
7-500-0008	LymphoTrack® MRD Software*	1 Software Package

These products are for Research Use Only (RUO). Not intended for diagnostic purposes.

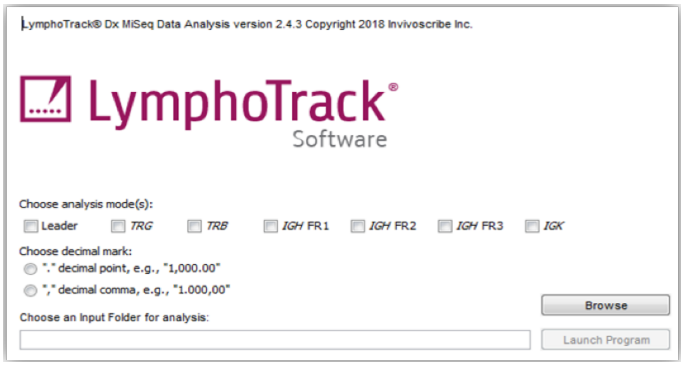
LymphoTrack® Bioinformatics Software

Software Use

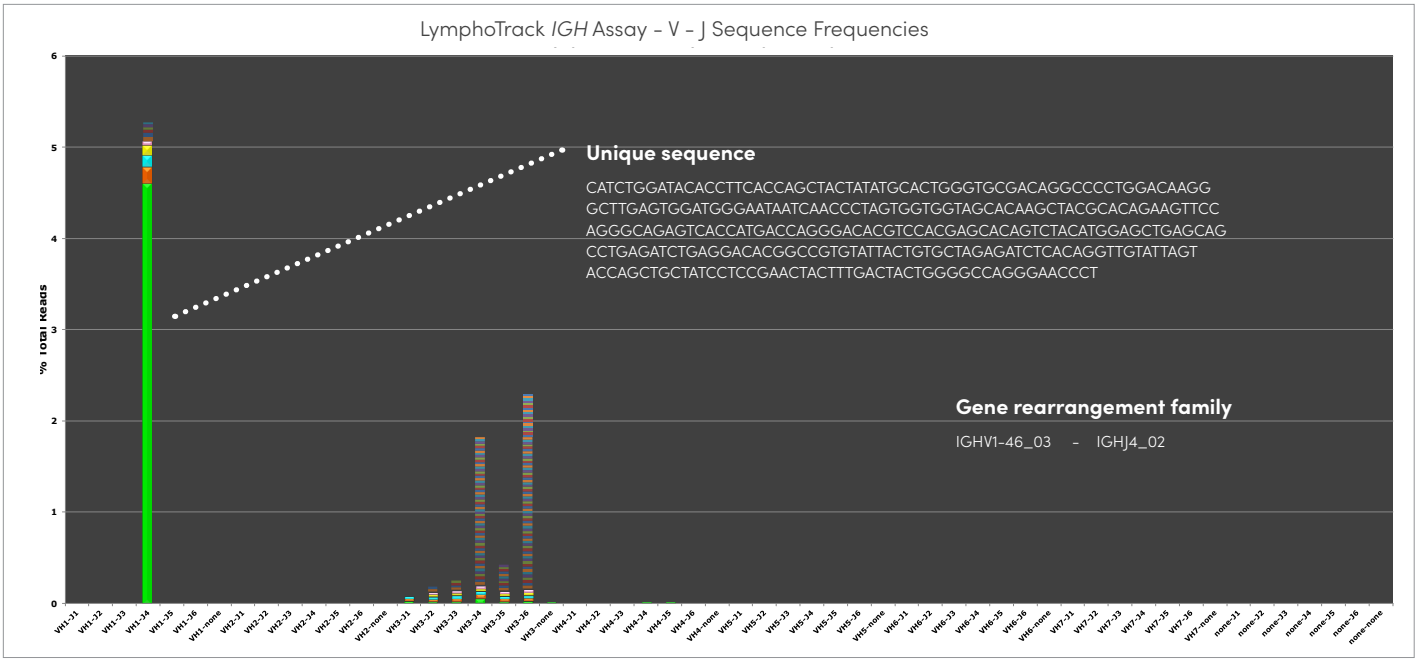
The LymphoTrack Bioinformatics Software package is used with LymphoTrack Assays to analyze raw FASTQ files for clonality analysis of single or multiple target data sets (*IGHV* Leader), *IGH* FR1, *IGH* FR2, *IGH* FR3, *IGK*, *TRG*, *TRB*). For data generated with LymphoTrack *IGHV* Leader or *IGH* FR1 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.



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.

Ordering Information

Catalog #	Products	Quantity Components
7-500-0009	LymphoTrack® Software- MiSeq™	1 Software Package
7-500-0007	LymphoTrack® Software - S5/PGM™	1 Software Package

These products are for Research Use Only (RUO). Not intended for diagnostic purposes.

LymphoTrack® Enterprise Software - MiSeq™

Software Use

When used with the LymphoTrack® Assays, the LymphoTrack® Enterprise Software enables fully automated pipeline bioinformatics data analysis for clonality using the raw FASTQ files from the Illumina® MiSeq™. The Dockerized Linux-based software is built for portability, flexibility and can easily be integrated into downstream reporting systems and/or LIMS.

Product Details

The LymphoTrack® Enterprise Software – MiSeq™ package was designed to enable automated pipelines for laboratories that generate high volumes of sequencing data using the LymphoTrack Assays. This Docker image enables clonal analysis of single or multiple targets (*IGHV* Leader, *IGH* FR1, *IGH* FR2, *IGH* FR3, *IGK*, *TRG* and/or *TRB*) and additionally provides the somatic hypermutation (SHM) status of samples run with the Leader or *IGH* FR1 assays. The FASTQ files that are output from a MiSeq™ sequencing run of samples prepared with LymphoTrack Assays, are automatically processed and output into a tab-separated value (.tsv) file using the LymphoTrack Enterprise Software. The analysis is initiated by either REST-API or command line interface. The data output can then be visualized in Microsoft Excel or imported into custom reports.

Ordering Information

Catalog #	Products	Quantity Components
INQUIRE	LymphoTrack® Enterprise Software- MiSeq™	1 Software Package

This product is for Research Use Only (RUO). Not intended for diagnostic purposes.

Measurable Residual Disease (MRD) Solutions Research Use Only (RUO)

MRD testing with Next-Generation Sequencing is a proven tool that may be used to develop hematologic malignancy management strategies.

LEARN MORE:
catalog.invivoscribe.com

Introduction

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}. The LymphoTrack MRD solution includes assays, controls and software to facilitate objective MRD assessments over time.

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 objective 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 enables objective monitoring of clonality over time and test standardization. Invivoscribe now offers both 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 the software to convert percent clonal reads into estimated clonal cell equivalents.

Key Benefits

- Complete solution for MRD
- Ensures test sensitivity to enable confidence in reporting
- Facilitates standardization of clonotype tracking
- Longitudinal assessment of mutation status of *FLT3* ITD, *NPM1,* B- and T-cell gene rearrangements and somatic hypermutation (SHM)
- LymphoTrack Assays formatted for both Illumina® and Thermo Fisher® NGS Platforms

Chapter Contents

RUO MRD Assays

References

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

LymphoTrack® MRD Software

Software Use

The LymphoTrack MRD Bioinformatics Software package may be purchased 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 be used to Create, Save and Load projects to objectively track up to 5 clonal sequences to monitor for disease relapse and for use in drug development studies.

The provided software is composed of four distinct parts:

1. A Project tool that can be used to plan experiments with sufficient confidence based on read depth, replicate count, and DNA input. Projects can also be Saved and Loaded for use in subsequent time points.

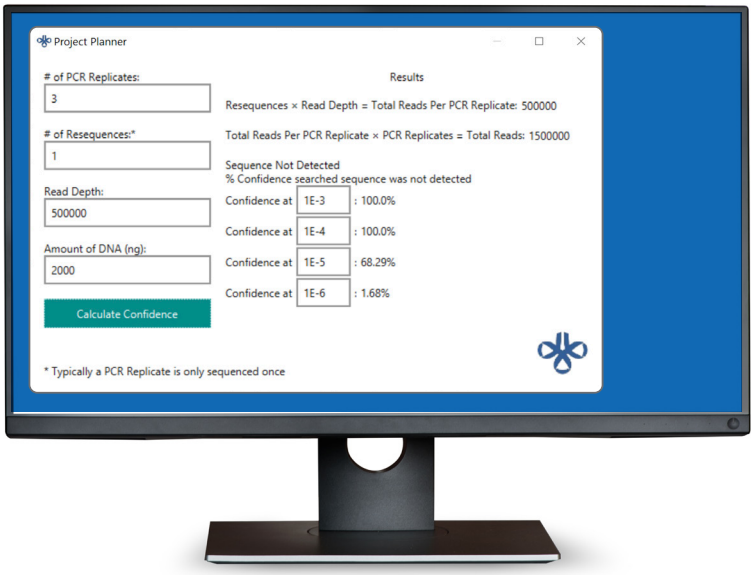
2. A bioinformatics data analysis application.

3. A PDF Sample Report identifying the clonal sequence(s) if present, a summary of the degree of mismatches, calculations of the read frequency and the degree of confidence if clonal sequence(s) are not detected at various sensitivities.

4. A PDF Summary Report that will automatically generate longitudinal graphs of up to 5 clonal frequencies for a Subject and summary tables with estimates of clonal cell equivalents and clonal frequencies for all queried sequences if LymphoQuant Internal Controls is used.

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.



LymphoTrack MRD Sample 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 #3 Details for Subject 1 for Collection/Timepoint: 2020/06/03

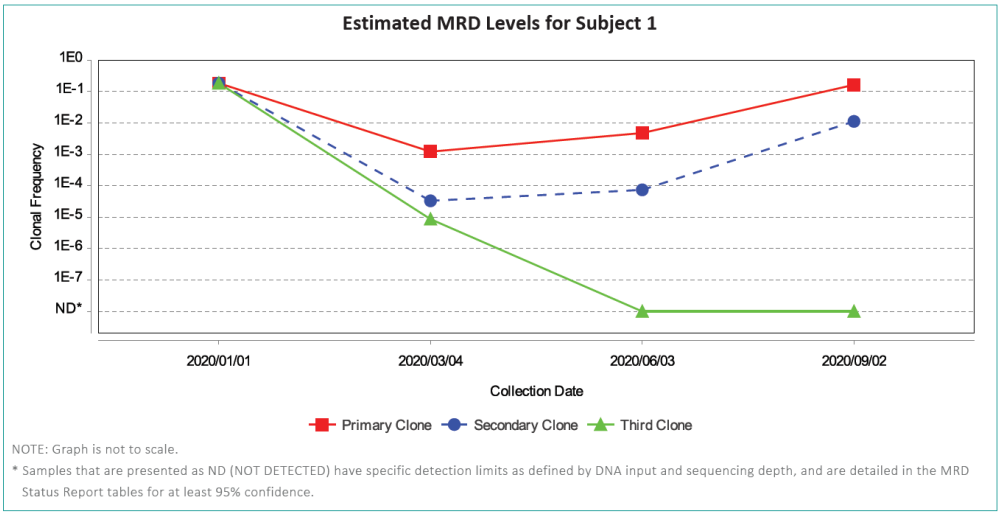
Sequence Name	PCR Replicate(s)	Total Reads	Gene Target	MRD Result
Third Clone	1	762940	IGH FR1	NOT DETECTED

GCGTCTGGATTCATTTTCCCTAATGGACAGCCTGAGAGCCGAGGACACGGGTGTGTATAAGTGTGCGAGAAATAGCGTGATGGAAATGCGTGCTTCGTGGTGTCTGGGGCATAG
GAGCCAC

PCR Replicate Details	Cumulative Target Read Count	Cumulative % Total Reads	Cumulative LymphoQuant Read Count	Clonal Frequency
Exact Match	0	0%	100	0
1 Mismatch	1	0.0002%	103	0
2 Mismatch	1	0.0002%	105	0

Detection Limit	% Confidence	Detection Limit	% Confidence
1E-3	99.999%	1E-5	53.761%
1E-4	99.999%	1E-6	2.004%

MRD Summary Graph



NOTE: Graph is not to scale.

* Samples that are presented as ND (NOT DETECTED) have specific detection limits as defined by DNA input and sequencing depth, and are detailed in the MRD Status Report tables for at least 95% confidence.

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

Ordering Information

Catalog #	Products	Quantity Components
7-500-0008	LymphoTrack® MRD Software**	1 Software Package

** 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 (RUO). Not intended for diagnostic purposes.

FLT3 ITD MRD Assay

Assay Use

This Research Use Only (RUO) assay identifies internal tandem duplications (ITD) of the *FLT3* gene.

Summary and Explanation of the Test

FLT3 ITD MRD is an NGS-based assay for use on the Illumina® MiSeq™. *FLT3* ITD MRD Assay tracks internal tandem duplication mutations of the *FLT3* gene to identify measurable residual disease in subjects following acute myeloid leukemia diagnosis (AML) and treatment. The assay is an amplicon-based approach which uses polymerase chain reaction to amplify the region of interest and next-generation sequencing to detect the region of interest.

The assay kit includes 24 master mixes, along with a ready to use positive and negative controls. Primers included in the master mix are designed with Illumina® adapters containing unique dual-indexes allowing for multiplexing at two levels. First, the multiplexing of up to 21 unique individuals in parallel in a single sequencing run, and second, the multiplexing of multiple targets in a single sequencing run.

The *FLT3* ITD MRD Assay is for use with the Illumina® MiSeq™ platform. It was designed to produce sequencing data that can be analyzed using the *FLT3* ITD MRD Software package. The *FLT3* ITD MRD Software is a Dockerized Linux-based package built for portability, flexibility, and can easily be integrated into downstream reporting system and/or LIMS. It provides automated bioinformatic analyses of raw FASTQ files generated by the Illumina® MiSeq™, when used with the *FLT3* ITD MRD Assay. Individual sample and aggregate sample data output is generated in under an hour in the form of .tsv (tab-separated value) files.

This deep-sequencing assay can detect internal tandem duplications up to 252 bp at an RUO validated allelic sensitivity of $5x10^{-5}$.

Background

FLT3 mutations are one of the most common mutations in AML, occurring in roughly ~30% of adult AML subjects.[1] The internal tandem duplication is the most common *FLT3* mutation and is present in about 25% of AML subjects.[1,2] *FLT3* ITD is considered a driver mutation at diagnosis and in relapsed AML,[1,3] conferring a higher relapse rate and poor prognosis.[1] Since *FLT3* ITD mutations are present in 75% of relapsed subjects which were *FLT3* ITD-mutated at diagnosis,[3] ITDs can serve as a biomarker to monitor for hematological disease progression and can guide therapeutic decisions.

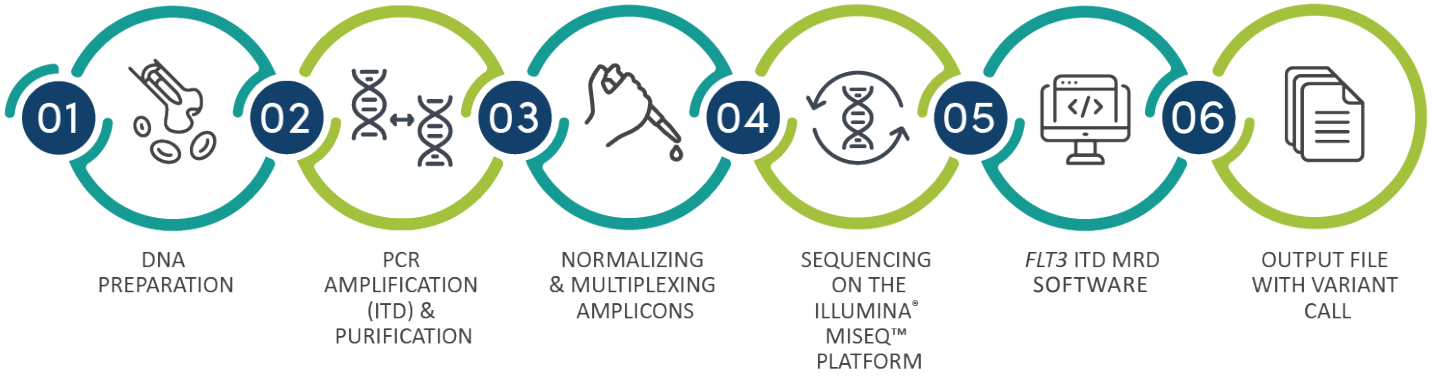
Specimen Requirements

700 ng DNA isolated from whole blood or bone marrow.

References

1. Levis, M. *Hematology Am Soc Hematol Educ Program* (2013) 2013:220-6.
2. Daver, N. et al., *Leukemia* (2019) 33:299-312.
3. Krönke, J. et al., *Blood* (2013) 122(1):100-8.

Figure 1. ***FLT3*** **ITD MRD Assay Workflow**



Reagents

Dual-indexed Master Mix	Index # (I7 Index ID)	Index # (I5 Index ID)
FLT3 ITD IA13	N701	N502
FLT3 ITD IA14	N702	N504
FLT3 ITD IA15	N703	N506
FLT3 ITD IA16	N704	N508
FLT3 ITD IA17	N705	N501
FLT3 ITD IA18	N706	N503
FLT3 ITD IA19	N707	N505
FLT3 ITD IA20	N708	N507
FLT3 ITD IA21	N709	N502
FLT3 ITD IA22	N710	N504
FLT3 ITD IA23	N711	N506
FLT3 ITD IA24	N712	N508
FLT3 ITD IB01	N702	N501
FLT3 ITD IB02	N703	N503
FLT3 ITD IB03	N704	N505
FLT3 ITD IB04	N705	N507
FLT3 ITD IB05	N706	N502
FLT3 ITD IB06	N707	N504
FLT3 ITD IB07	N708	N506
FLT3 ITD IB08	N709	N508
FLT3 ITD IB09	N710	N501
FLT3 ITD IB10	N711	N503
FLT3 ITD IB11	N712	N505
FLT3 ITD IB12	N701	N507

Controls	Concentration	Units in 96 Reaction Assay
FLT3 ITD MRD POS (+)	20 ng/µL	2 x 500 µL tube
FLT3 ITD MRD NEG (-)	20 ng/µL	2 x 500 µL tube

Ordering Information

Catalog #	Products	Quantity Components
1-412-0019	*FLT3* ITD Assay	24 indices - 4 sequencing reactions each
1-412-0029	*FLT3* ITD MRD Software	1 Software Package

These products are for Research Use Only (RUO). Not intended for diagnostic purposes.

NPM1 MRD Assay

Assay Use

This Research Use Only (RUO) assay identifies four base pair insertions of the *NPM1* gene.

Summary and Explanation of the Test

NPM1 MRD is an NGS-based assay for use on the Illumina® MiSeq™. *NPM1* MRD Assay tracks four base pair insertions in the *NPM1* gene to identify measurable residual disease in subjects following acute myeloid leukemia diagnosis (AML) and treatment. The assay is an amplicon-based approach which uses polymerase chain reaction to amplify the region of interest and next-generation sequencing to detect the region of interest.

The assay kit includes 24 master mixes, along with a ready to use positive and negative controls. Primers included in the master mix are designed with Illumina® adapters containing unique dual-indexes allowing for multiplexing at two levels. First, the multiplexing of up to 21 unique individuals in parallel in a single sequencing run, and second, the multiplexing of multiple targets in a single sequencing run.

The *NPM1* MRD Assay is for use with the Illumina® MiSeq™ platform was designed to produce sequencing data that can be analyzed using the *NPM1* MRD Software package. The *NPM1* MRD Software is a Dockerized Linux-based package built for portability, flexibility, and can easily be integrated into downstream reporting system and/or LIMS. It provides automated bioinformatic analyses of raw FASTQ files generated by the Illumina® MiSeq™, when used with the *NPM1* MRD Assay. Individual sample and aggregate sample data output is generated in under an hour in the form of .tsv (tab-separated value) files.

This deep-sequencing assay can detect four base mutation insertions characterized as 'A', 'B', 'D', or 'Other' at an RUO validated allelic sensitivity of $5x10^{-5}$.

Background

One of the most commonly mutated genes in AML, *NPM1* gene mutations occur in about one-third of the cases of primary AML in adults.[1] While risk stratification of *NPM1*-mutated AML is ever evolving,[2] *NPM1* gene mutations are common,[1] AML specific,[3] and its mutant transcripts are expressed at high levels,[2] making the mutation an ideal target for monitoring measurable residual disease[2] to guide therapeutic decisions.

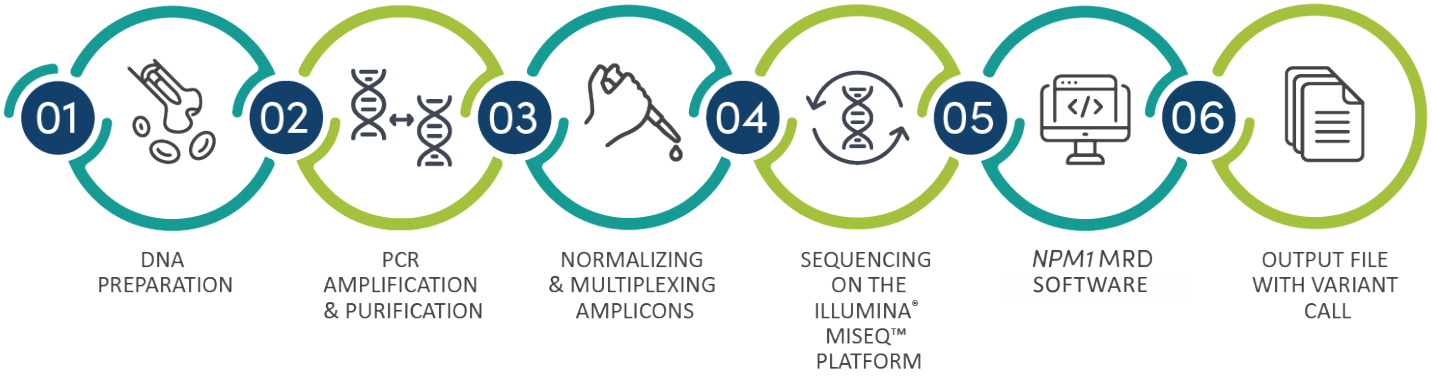
Specimen Requirements

700 ng DNA isolated from whole blood or bone marrow.

References

1. Falini, B. et al., *N Engl J Med.* (2005) 352:254-266.
2. Falini, B. et al., *Leukemia* (2021) 35:3113-3126.
3. Liso, A. et al., *Leukemia* (2008) 22:1285–1289.

Figure 1. *NPM1* MRD Assay Workflow



Reagents

Dual-indexed Master Mix	Index # (I7 Index ID)	Index # (I5 Index ID)
NPM1 MRD IA01	N701	N501
NPM1 MRD IA02	N702	N503
NPM1 MRD IA03	N703	N505
NPM1 MRD IA04	N704	N507
NPM1 MRD IA06	N706	N504
NPM1 MRD IA07	N707	N506
NPM1 MRD IA08	N708	N508
NPM1 MRD IA09	N709	N501
NPM1 MRD IA10	N710	N503
NPM1 MRD IA11	N711	N505
NPM1 MRD IA12	N712	N507
NPM1 MRD IA25	N705	N502
NPM1 MRD IB13	N702	N502
NPM1 MRD IB14	N703	N504
NPM1 MRD IB15	N704	N506
NPM1 MRD IB16	N705	N508
NPM1 MRD IB17	N706	N501
NPM1 MRD IB18	N707	N503
NPM1 MRD IB19	N708	N505
NPM1 MRD IB21	N710	N502
NPM1 MRD IB22	N711	N504
NPM1 MRD IB23	N712	N506
NPM1 MRD IB24	N701	N508
NPM1 MRD IB26	N709	N507

Controls	Concentration	Units in 96 Reaction Assay
NPM1 MRD POS (+)	20 ng/µL	2 x 500 µL tube
NPM1 MRD NEG (-)	20 ng/µL	2 x 500 µL tube

Ordering Information

Catalog #	Products	Quantity Components
1-416-0019	*NPM1* MRD Assay	24 indices - 4 sequencing reactions each
1-416-0029	*NPM1* MRD Software	1 Software Package

These products are for Research Use Only (RUO). Not intended for diagnostic purposes.

IdentiClone®

Capillary - CE-IVD Assays

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

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Notice
This product is covered by one or more patents licensed to Invivoscribe Inc, including European Patent No. 1549764 and 2418287 (each validated in 16 countries), European Patent No. 2460889, Japanese Patent No. 4708029, United States Patent No. 8859748 and United States Patent No. 10280462.

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.

Introduction

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

The Invivoscribe CE-marked IdentiClone Assays represent a simple, cost-effective 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 performed at more than thirty prominent independent testing centers throughout Europe in a collaborative study known as the BIOMED-2 Concerted Action.[1] These PCR-based tests include standardized Instructions For Use (IFUs) with interpretation guidelines describing the use of master mixes and controls. The same thermal cycler program and similar detection methods are used for all IdentiClone kits to improve consistency, reduce human error, and facilitate cross-training.

Chapter Contents

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

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.

IdentiClone® *IGH* + *IGK* B-Cell Clonality Assays

Assay Description

The IdentiClone *IGH* + *IGK* B-Cell Clonality Assay is an *in vitro* diagnostic product intended for PCR-based detection of clonal immunoglobulin heavy chain and kappa light chain gene rearrangements in patients with suspect lymphoproliferations. Specifically, the *IGH* + *IGK* B-Cell Clonality Assay can be used to:

- Identify clonality in atypical lymphoproliferative disorders
- Support a differential diagnosis between reactive lesions and hematologic malignancies[4]
- 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

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 (V_H) region, and the joining (J_H) 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.

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

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

PCR/SB concordance:[2]		PCR sensitivity:	SB sensitivity:
IGH + *IGK*:	85%	98%	39%

References

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).

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

IdentiClone®

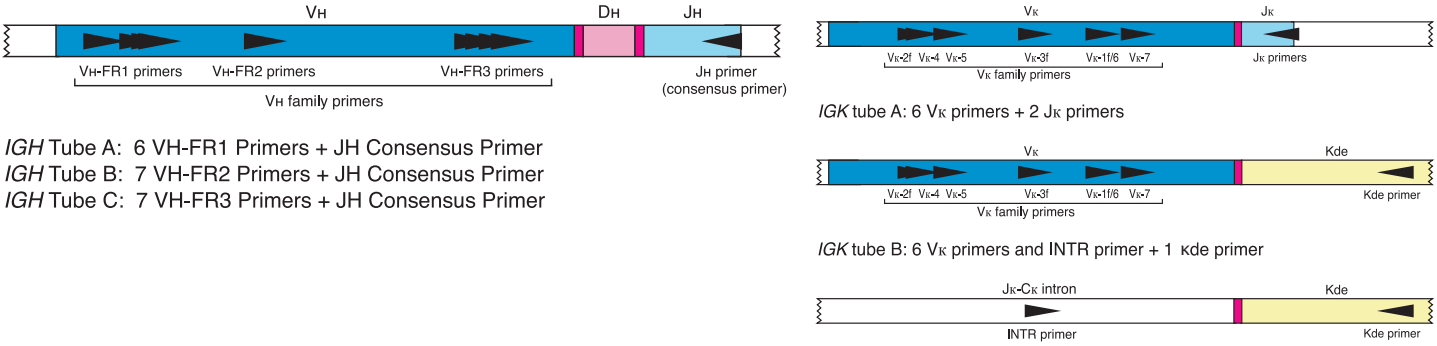


Figure Legend: Simple representation of the organization of a rearranged immunoglobulin heavy chain (*IGH*) gene on chromosome 14q32.33 and the immunoglobulin kappa light chain gene on chromosome 2p11.2. Black arrows represent the relative positions of primers that target the conserved framework regions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

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}, 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

Ordering Information

Catalog #	Products	Quantity
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 available in regions that accept CE-IVD products.

IdentiClone® *IGH* Gene Clonality Assays

Assay Description

The IdentiClone *IGH* Gene Clonality Assay is an *in vitro* diagnostic product intended for PCR-based detection of clonal immunoglobulin heavy chain gene rearrangements in patients with suspect lymphoproliferations.

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

- Identify clonality in atypical lymphoproliferative disorders
- Support a differential diagnosis between reactive lesions and hematologic malignancies[4]
- 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

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 (V_H) region and the joining (J_H) 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.

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

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

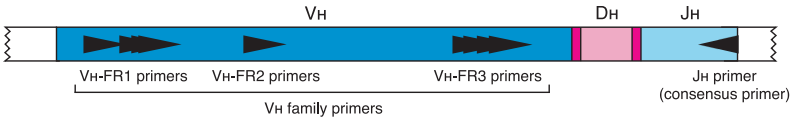
PCR/SB concordance:[2]		PCR sensitivity:	SB sensitivity:
IGH + *IGK*:	85%	98%	39%

References

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

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

IdentiClone®



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

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 J_H 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.

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 + 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
IGH Tube D	D_H1-6 + J_H	1 x 1500 µL tube	10 x 1500 µL tubes
IGH Tube E	D_H7 + J_H	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

Ordering Information

Catalog #	Products	Quantity
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 available in regions that accept CE-IVD products.

IdentiClone® *IGK* Gene Clonality Assays

Assay Description

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

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

- Identify clonality in atypical lymphoproliferative disorders
- Support a differential diagnosis between reactive lesions andhematologic 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

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.

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]
IGK: 90% sensitivity / 90% specificity

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

PCR/SB concordance:[2]		PCR sensitivity:	SB sensitivity:
IGH + *IGK*:	85%	98%	39%

References

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

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

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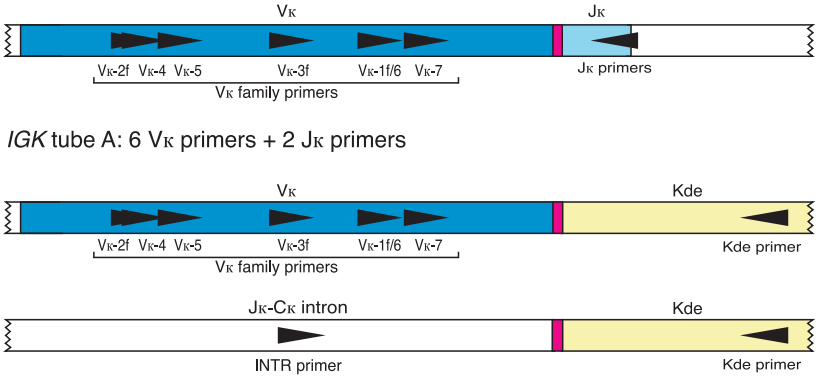


IGK tube A: 6 Vκ primers + 2 Jκ primers

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

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.

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}, 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

Ordering Information

Catalog #	Products	Quantity
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 available in regions that accept CE-IVD products.

IdentiClone® *IGL* Gene Clonality Assays

Assay Description

The IdentiClone *IGL* Gene Clonality Assay is an *in vitro* diagnostic product intended for PCR-based detection of clonal immunoglobulin lambda light chain gene rearrangements in patients with suspect lymphoproliferations and can be used to:

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

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.

Performance Characteristics

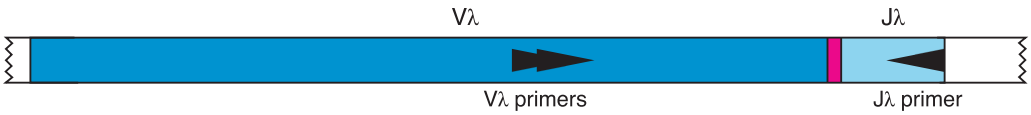
Data from two independent studies that tested more than 300 patient samples of varying types suggests the diagnostic accuracy of selected IdentiClone tests to be 96%. In both peer-reviewed studies, there were no clear false-positive results generated using the IdentiClone tests, and there was a high 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]
IGL:	86% sensitivity / 92% specificity

References

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

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 VλJ1, 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.

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

Ordering Information

Catalog #	Products	Quantity
9-103-0011	IdentiClone® *IGL* Gene Clonality Assay - ABI Fluorescence Detection	33 reactions

These are *in vitro* diagnostic products and are available in regions that accept CE-IVD products.

IdentiClone® *TCRB* Gene Clonality Assays

Assay Description

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

Specifically, the *TCRB* Gene Clonality Assay 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

These kits include four master mixes. *TCRB* Tubes A and B target framework regions within the variable region, and the joining region (V_β) of the *TCR* beta chain locus. *TCRB* Tube C targets the diversity and joining (J_β) 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.

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]
TRB:	86% sensitivity / 98% specificity

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

	PCR/SB concordance:[2]	PCR sensitivity:	SB sensitivity:
TRB:	85% 96%	35%	

References

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

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

IdentiClone®

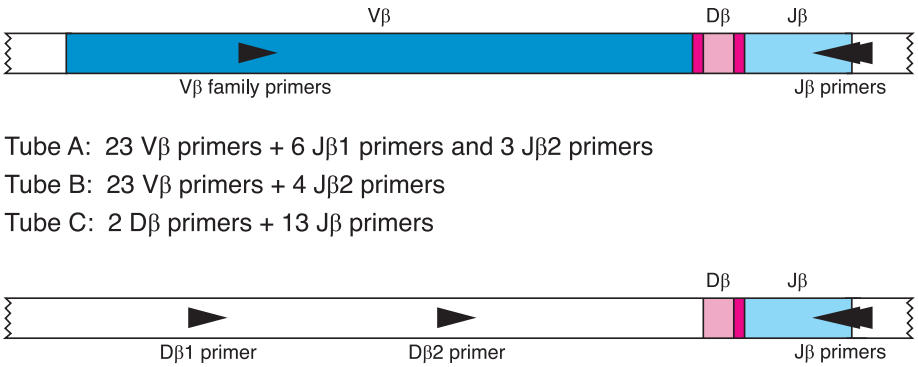


Figure Legend: Simplified diagram of a representative rearranged T-cell receptor beta gene showing the approximate placement of the upstream and downstream DNA primers. The numbers of primers and their specificity are listed for Master Mix Tubes A, B, and C.

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

Ordering Information

Catalog #	Products	Quantity
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 available in regions that accept CE-IVD products.

IdentiClone® 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 suspect 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]

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.

References

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 is based on the EuroClonality/BIOMED-2 Concerted Action BMH4-CT98-3936.
The performance of this assay was reviewed and validated by the EuroClonality/BIOMED-2 Group.[2]

IdentiClone®

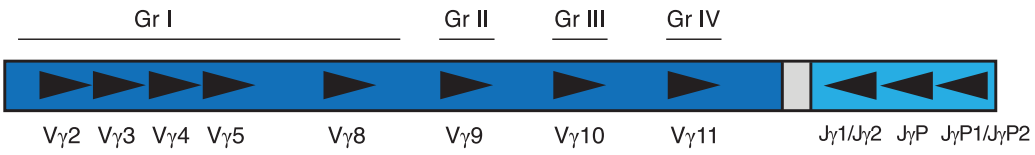


Figure Legend: Simple representation of the organization of the T-cell receptor gamma gene on chromosome 7p14. Black arrows represent the relative positions of primers that target the variable region genes and the downstream joining region gene segments that are involved in rearrangements in T-cell lymphomas. 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.

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

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 available in regions that accept CE-IVD products.

IdentiClone® *TCRD* Gene Clonality Assays

Assay Description

The IdentiClone *TCRD* Gene Clonality Assay is an *in vitro* diagnostic product intended for PCR-based detection of clonal T-cell receptor delta chain gene rearrangements in patients with suspect lymphoproliferations and 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

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.

Performance Characteristics

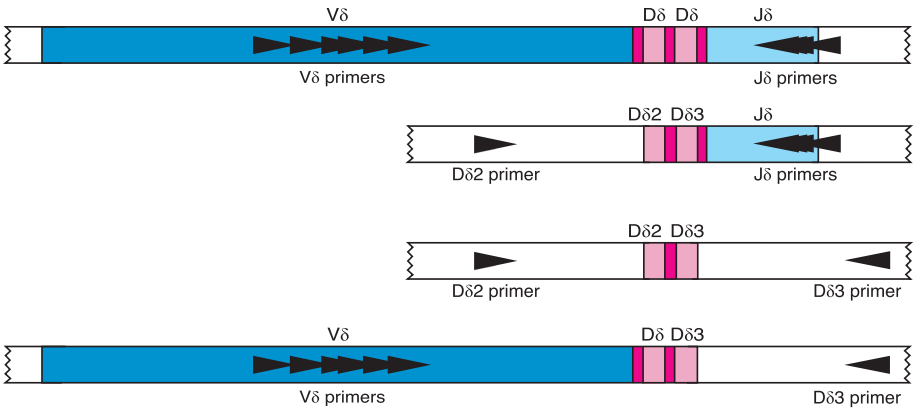
Data from an independent, peer-reviewed study suggests the diagnostic accuracy of selected IdentiClone tests to be 96%. There were no clear false-positive results generated using the IdentiClone tests, and there was a high level of precision. The clinico- histopathological diagnosis correlates well with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

	PCR/SB concordance:[1]
TRD:	83% sensitivity / 95% specificity

Reference

1. 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.



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

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.

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

Ordering Information

Catalog #	Products	Quantity
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 available in regions that accept CE-IVD products.

LeukoStrat®

Capillary Detection – CE-IVD Assays and IVD Companion Diagnostic Assays

The IVDR Approved LeukoStrat CDx *FLT3* Mutation Assay will be commercially available Summer 2023.

LeukoStrat Assay Kits are *in vitro* diagnostic products intended for PCR-based detection of *FLT3* activating mutations (ITD, TKD) in patients with acute myelogenous leukemia (AML) using capillary electrophoresis methods, and include companion diagnostic assays.

LEARN MORE:
catalog.invivoscribe.com

Introduction

All tests include PCR master mixes and controls for ITD and TKD detection, along with Instructions For Use (IFUs). 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.

Companion Diagnostics

The LeukoStrat CDx *FLT3* Mutation Assays are companion diagnostics, and provide a complete solution with software automated PDF reports including mutant identification, mutant to wild type signal ratios, and clinical interpretation. Additional reagents of Taq DNA Polymerase (US, JP), EcoRV Enzyme (US, JP), and NEBuffer r3.1 (US, JP) are included in IVD kits.

Chapter Contents

LeukoStrat Assays are *in vitro* diagnostic products. CE-IVD products are not available for sale or use within North America. IVD products are available for sale or use where registered. Refer to product page for respective detail. For more information regarding the research use only reagents, please see the Gel and Capillary Research Use Only Assays section.

LeukoStrat® *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:

1. Identify internal tandem duplications (ITD) in the *FLT3* gene
2. 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] Many studies in AML have shown that the presence of *FLT3* (fms related tyrosine kinase 3) activating mutations portends a poor prognosis making it an attractive target for treatment. For this reason, *FLT3* mutation testing is required to stratify disease and determine appropriate treatment options.

Using this assay, DNA is amplified via PCR with fluorophore labeled primers, TKD amplicon is enzymatically digested, and *FLT3* mutations are detected via capillary electrophoresis. This test kit includes 2 PCR master mixes, along with positive and negative controls for mutant detection. Each master mix (*FLT3* ITD Master Mix and *FLT3* D835 Master Mix) contains a fluorophore-labeled PCR primer set for the respective detection of internal tandem duplication or TKD mutation.

Performance Characteristics

This assay can reliably detect mutations comprising more than 5% of the total cell population. Also, as demonstrated herein, the LeukoStrat *FLT3* Mutation Assay 2.0 detects *FLT3* ITD and TKD mutations with excellent concordance to NGS methodologies (Table 1, Table 2).

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

Percentage Agreement	Discordance #	Concordance #	*95% LL
Negative PA 100%	0	119	96.9%
Postive 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

Percentage Agreement	Discordance #	Concordance #	*95% LL
Negative PA 100%	0	137	96.9%
Postive 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).

References

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)

Reagents

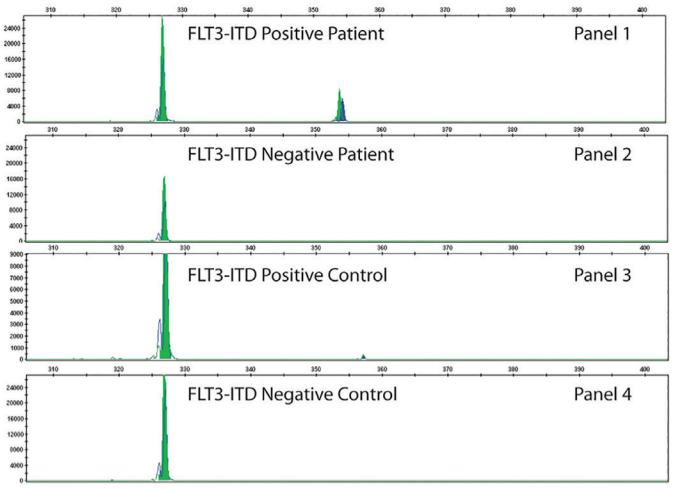
Controls	Concentration	Units in Assay	Units in MegaKit
FLT3 ITD Positive Control	50 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
FLT3 D835 Positive Control	50 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
FLT3 Negative Control	50 µg/mL	1 x 100 µL tube	5 x 100 µL tubes

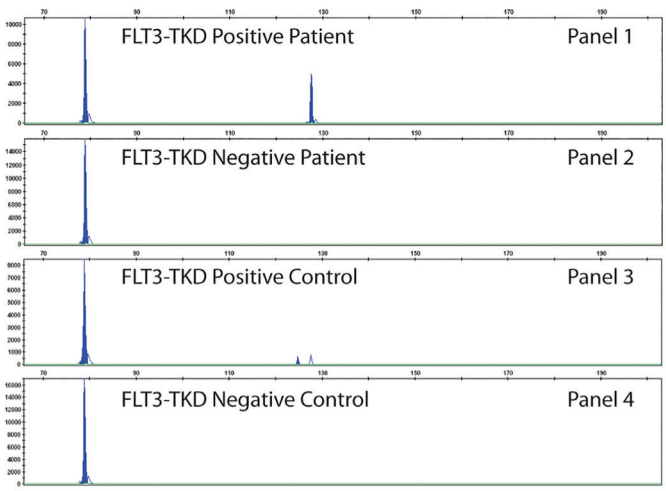
Master Mixes	Target	Units in Assay	Units in MegaKit
FLT3 ITD Master Mix – 6FAM & HEX	*FLT3* ITD	1 x 1500 µL tube	10 x 1500 µL tubes
FLT3 D835 Master Mix – 6FAM	*FLT3* TKD	1 x 1500 µL tube	10 x 1500 µL tubes

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

These are *in vitro* diagnostic products and are available in regions that accept CE-IVD products.

LeukoStrat® CDx *FLT3* Mutation Assay

FDA Approved Companion Diagnostic

Available in the USA - *In Vitro* Diagnostic Kit

FDA approved assay for assessment of acute myeloid leukemia (AML) patients eligible for treatment with RYDAPT® (midostaurin), XOSPATA® (gilteritinib), or VANFLYTA® (quizartinib), now available as US distributed kit. This *FLT3* companion diagnostic includes reagents along with software that identifies ITD and TKD mutations, generates mutant/wildtype signal ratios, and predicts response to gilteritinib, midostaurin, and quizartinib.

Clinical Significance of *FLT3* Mutation Status: Each year approximately 21,000 patients in the United States are diagnosed with AML. Of those diagnosed with AML, ~1 out of 3 are expected to have presence of *FLT3* mutations, (*FLT3*mut+). Since *FLT3*mut+ AML is clinically actionable, stratification of AML patients by testing for *FLT3* mutation status has become a standard of care.

Intended Use

The LeukoStrat CDx *FLT3* Mutation Assay is a PCR-based in vitro diagnostic test designed to detect internal tandem duplication (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).

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.

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

The LeukoStrat CDx *FLT3* Mutation Assay is used as an aid in the assessment of patients with *FLT3*-ITD+ AML for whom VANFLYTA® (quizartinib) treatment is being considered.

The test is for use on the 3500xL Dx Genetic Analyzer.

Summary and Explanation of the Test

Acute myelogenous leukemia (AML) in general has a poor prognosis. Many studies in AML have shown that the presence of *FLT3* activating mutations portends a poor prognosis making it an attractive target for treatment.[1,2]

The LeukoStrat CDx *FLT3* Mutation Assay targets regions of the *FLT3* gene to identify internal tandem duplication (ITD) mutations and tyrosine kinase domain (TKD) mutations, such as the D835 and I836 mutations.

The LeukoStrat CDx *FLT3* Mutation Assay includes reagents, software and procedures for isolating mononuclear cells and extracting DNA from patient peripheral blood or bone marrow 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

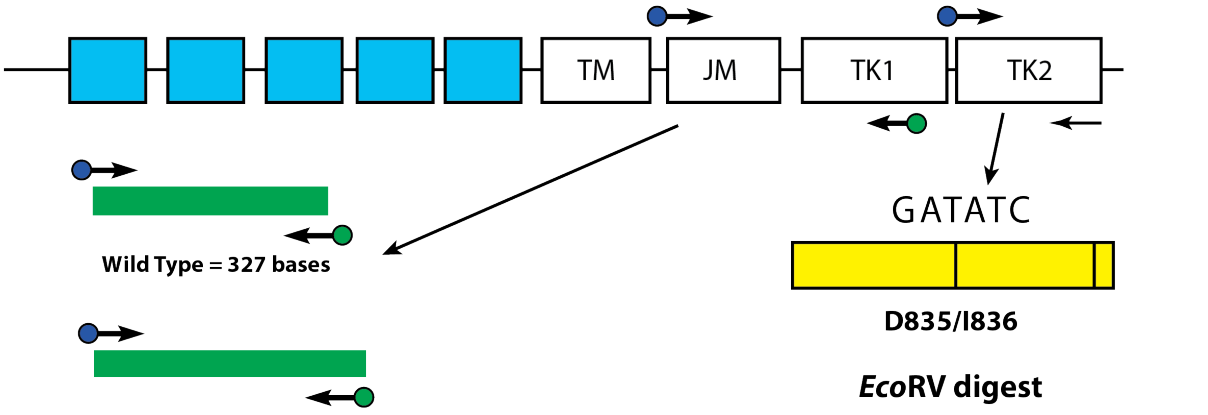
ITD Mutations of *FLT3*

The LeukoStrat CDx *FLT3* Mutation Assay uses fluorescently labeled primers that are in and around 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 327±1 bp (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 (see Figure, right).

Method Schematic: *FLT3* ITD and TKD Mutant Detection



***Eco*RV digest**

Undigested = 145 bases or 147 bases
Wild Type digested = 79 bases
Mutant digested, point mutation = 127 bases
Mutant digested, deletion = 124 bases

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	1 x 200 µL tube
EcoRV Enzyme	1 x 200 µL tube
NEBuffer r3.1	1 x 1250 µL tube

Ordering Information

Catalog #	Products	Quantity
K-412-0361	LeukoStrat® CDx *FLT3* Mutation Assay	33 reactions
K-412-0371	LeukoStrat® CDx *FLT3* Software	CD complimentary with purchase
K-412-0401	LeukoStrat® CDx Assay Installer	USB complimentary with purchase

References

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).

These are *in vitro* diagnostic (IVD) products, and are available for sale or use in the United States only

LeukoStrat® CDx *FLT3* Mutation Assay

CE-marked Companion Diagnostic

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*

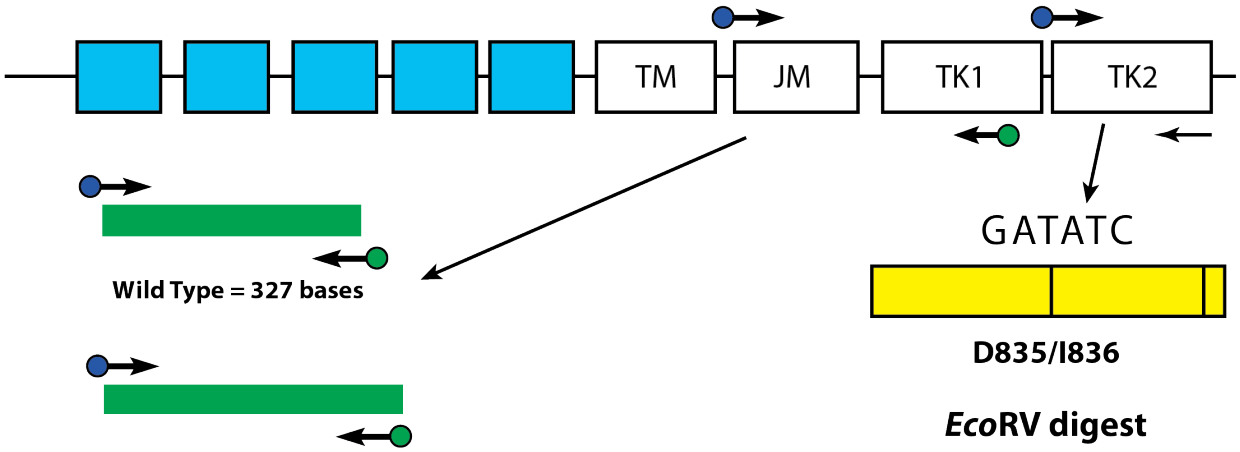
The LeukoStrat CDx *FLT3* Mutation Assay uses fluorescently labeled primers that are in and around the JM region. Wild-type *FLT3* alleles will amplify and produce a product at 327±1 bp as measured by this assay, while alleles that contain ITD mutations will produce a product that exceeds 327±1 bp (see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

References

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).



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

Ordering Information

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

These are *in vitro* diagnostic products and are available in regions that accept CE-IVD products.

LeukoStrat® CDx *FLT3* Mutation Assay

PMDA/MHLW Approved Companion Diagnostic

Available in the Japan

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 with fluorescently labeled primers, amplicon is enzymatically digested (TKD), 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 and around 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 327±1 bp (see Figure, right).

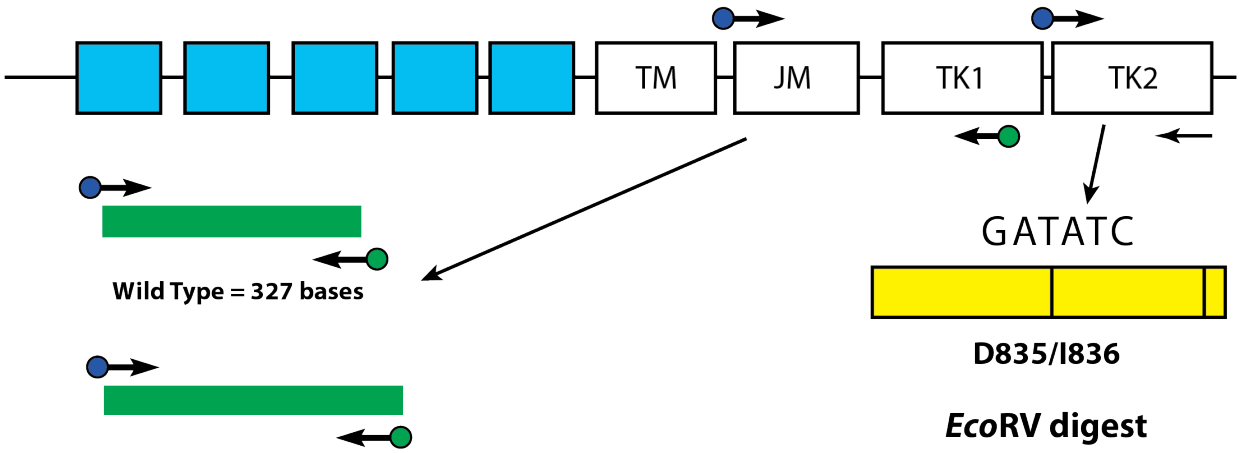
TKD Mutations of *FLT3*

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 (see Figure, right).

References

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



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
NEBuffer r3.1	1 x 1250 µL tube

Ordering Information

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

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

LeukoStrat®
リューコストラット CDX FLT3 変異検査

Gel and Capillary

Research Use Only (RUO) Assays

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

LEARN MORE:
catalog.invivoscribe.com

Introduction

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). Select assays are also available in 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

Chapter Contents

IGH + *IGK* B-Cell Clonality Assays

Assay Use

Immunoglobulin heavy chain (*IGH*) and Kappa light chain (*IGK*) gene clonality assays are useful for identification of B-cell clonality, studying clonal B-cell populations, and 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 (VH) region and the joining (JH) region of the *IGH* locus. *IGK* tubes A and B target the variable (Vκ), intragenic and joining (Jκ), 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 (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 VH3, VH4, and VH1 families, which together cover 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]

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 (Vκ) gene segments belonging to seven subgroups, five 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[2]; 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.

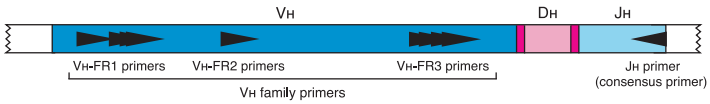
Specimen Requirements

This assay tests extracted and purified genomic DNA (gDNA)

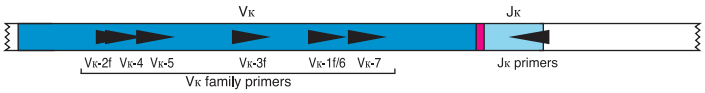
References

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).

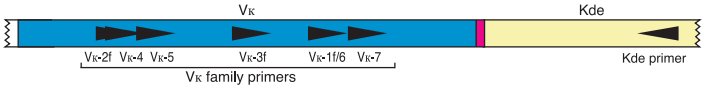
EuroClonality This assay is based on the EuroClonality/BIOMED-2 Concerted Action BMH4-CT98-3936.



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 VK primers + 2 JK primers



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

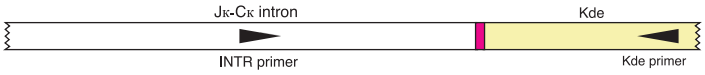


Figure Legend: Simple representation of the organization of a rearranged immunoglobulin heavy chain (*IGH*) gene on chromosome 14 and the immunoglobulin kappa light chain gene on chromosome 2p11.2. Black arrows represent the relative positions of primers that target the conserved framework regions (FR1-3)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

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

Ordering Information

Catalog #	Products	Quantity
1-100-0010	*IGH* + *IGK* B-Cell Clonality Assay - Gel Detection	33 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

These products are for Research Use Only (RUO).
Not intended for diagnostic purposes.

IGH Gene Rearrangement Assays

Assay Use

Immunoglobulin heavy chain (*IGH*) gene clonality assays are useful for the study of identifying clonal B-cell populations and 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 (J_H) 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. PCR products can be analyzed by capillary electrophoresis. 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 (V_H), diversity (D_H), and joining (J_H) 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]

Specimen Requirements

This assay tests extracted and purified genomic DNA (gDNA).

References

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).

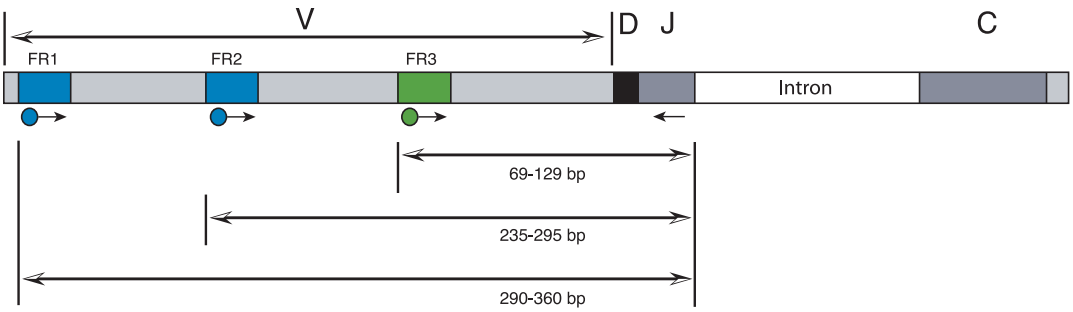


Figure Legend: Genomic organization of a rearranged immunoglobulin heavy chain gene on chromosome 14. The blue and green arrows represent primers targeting the conserved framework regions within the variable region gene. The relative location, size range of valid products, and colors correspond to the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

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 + J_H	1 x 1500 µL tube	10 x 1500 µL tubes
IGH Framework 2	Framework 2 + J_H	1 x 1500 µL tube	10 x 1500 µL tubes
IGH Framework 3	Framework 3 + J_H	1 x 1500 µL tube	10 x 1500 µL tubes
Amplification Control	*HLA-DQa*	1 x 1500 µL tube	10 x 1500 µL tubes

Ordering Information

Catalog #	Products	Quantity
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

These products are for Research Use Only (RUO).
Not intended for diagnostic purposes.

IGH Gene Clonality Assays

Assay Use

Immunoglobulin heavy chain (*IGH*) clonality assays are useful for identification of B-cell clonality, studying clonal B-cell populations, and evaluation of new research and methods in malignancy studies.

Summary and Explanation of the Test

Five master mixes target conserved regions within the variable (V_H), diversity (D_H), and the joining (J_H) 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 J_H region primer. Tube B contains seven framework region 2 (FR2) primers and a consensus J_H primer. Tube C contains seven framework region 3 (FR1) primers and a consensus J_H primer. Tube D contains six D_H region primers and a consensus J_H region primer. Tube E contains a D_H7 region primer and a consensus J_H 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 (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 the V_H3, V_H4, and V_H1 family, together covering 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] In addition to V_H-J_H rearrangements, incomplete D_H-J_H rearrangements have been found in mature and immature B-cell malignancies. Therefore, D_H-J_H PCR analysis may be of added value for clonality assessment.[2]

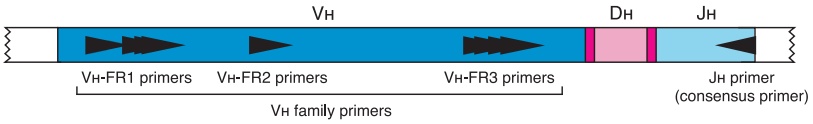
Specimen Requirements

This assay tests extracted and purified genomic DNA (gDNA).

References

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.



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

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 (D_H1-7) regions, and the downstream consensus J_H 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.

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 + 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
IGH Tube D	D_H1-6 + J_H	1 x 1500 µL tube	10 x 1500 µL tubes
IGH Tube E	D_H7 + J_H	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

Ordering Information

Catalog #	Products	Quantity
1-101-0020	*IGH* Gene Clonality Assay - Gel Detection	33 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

These products are for Research Use Only (RUO).
Not intended for diagnostic purposes.

IGK Gene Clonality Assays

Assay Use

Immunoglobulin kappa light chain (*IGK*) gene clonality assays are useful for the identification of B-cell clonality, studying clonal B-cell populations, and 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.

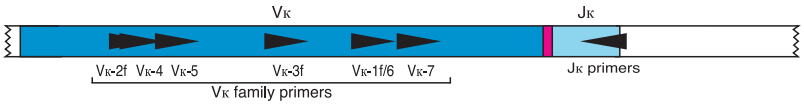
Specimen Requirements

This assay tests extracted and purified genomic DNA (gDNA).

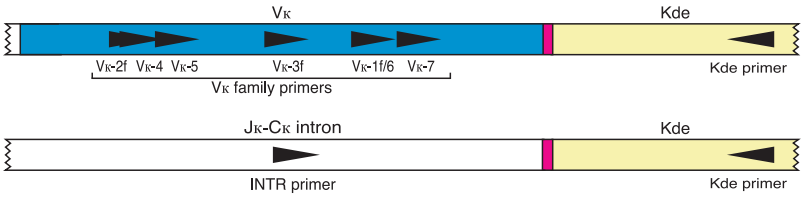
References

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.



IGK tube A: 6 Vκ primers + 2 Jκ primers



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

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.

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

Ordering Information

Catalog #	Products	Quantity
1-102-0020	*IGK* Gene Clonality Assay - Gel Detection	33 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

These products are for Research Use Only (RUO).
Not intended for diagnostic purposes.

IGL Gene Clonality Assays

Assay Use

Immunoglobulin lambda light chain (*IGL*) gene clonality assays are useful for the identification of B-cell clonality, studying clonal B-cell populations, and 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 (Vλ-Jλ) rearrangements potentially represent an attractive PCR target for clonality studies to compensate for false-negative *IGH* V_H-J_H PCR results mainly caused by somatic hypermutations. The limited size of the junctional region may create a challenge to distinguish polyclonal from monoclonal rearrangements when 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 also be used for analysis.[1]

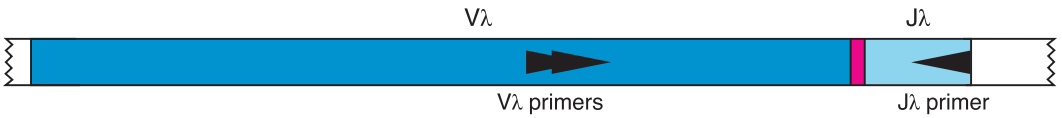
Specimen Requirements

This assay tests extracted and purified genomic DNA (gDNA).

References

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.

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

Ordering Information

Catalog #	Products	Quantity
1-103-0010	*IGL* Gene Clonality Assay - Gel Detection	33 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

These products are for Research Use Only (RUO).
Not intended for diagnostic purposes.

TCRB Gene Clonality Assays

Assay Use

T-Cell receptor beta (*TCRB*) gene clonality assays are useful for the identification of T-cell clonality, studying clonal T-cell populations, and 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]

Specimen Requirements

This assay tests extracted and purified genomic DNA (gDNA).

References

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.

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

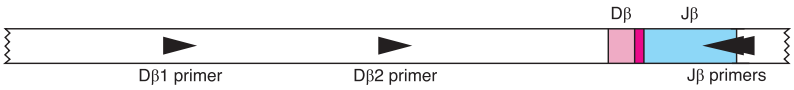


Figure Legend: Simplified diagram of a representative rearranged T-cell receptor beta gene showing the approximate placement of the upstream and downstream DNA primers. The numbers of primers and their specificity are listed for Master Mix Tubes A, B, and C.

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
Specimen Control Size Ladder	Multiple Genes	1 x 1500 µL tube	10 x 1500 µL tubes

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

These products are for Research Use Only (RUO).
Not intended for diagnostic purposes.

T-Cell Receptor Gamma Gene Rearrangement Assay 2.0

Assay Use

This Research Use Only assay identifies T-cell receptor gamma (*TCRG*) chain gene rearrangements and is useful for the identification of clonal T-cell populations and 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 contains a single multiplex master mix which includes primers for all known groups of *TCR* gamma variable (Vγ) region genes and joining (Jγ) region genes involved in rearrangements. In addition, positive and negative controls, as well as a Specimen Control Size Ladder Master Mix are included. PCR products can be analyzed by polyacrylamide or capillary electrophoresis detection.

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. This multiplex PCR assay represents an improvement over other 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, providing a more comprehensive analysis to include Vγ and Jγ regions that would not be identified with a single Vγ (1-8) and Jγ1/Jγ2 primer set.

In addition, 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 leads 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

This assay tests extracted and purified genomic DNA (gDNA).

References

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]

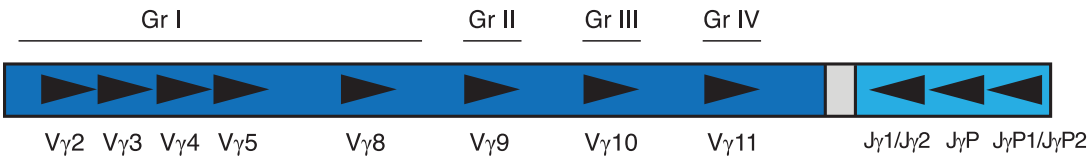


Figure Legend: Simple representation of the organization of the T-cell receptor gamma gene on chromosome 7. Black arrows represent the relative positions of primers that target the variable region genes and the downstream joining region gene segments that are involved in rearrangements in T-cell lymphomas. 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.

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

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

These products are for Research Use Only (RUO).
Not intended for diagnostic purposes.

T-Cell Receptor Gamma Gene Rearrangement Assays

Assay Use

T-Cell receptor gamma (*TCRG*) gene clonality assays are useful for the identification of T-cell clonality, studying clonal T-cell populations, and 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. PCR products can be analyzed by capillary electrophoresis.

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

This assay tests extracted and purified genomic DNA (gDNA).

References

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).

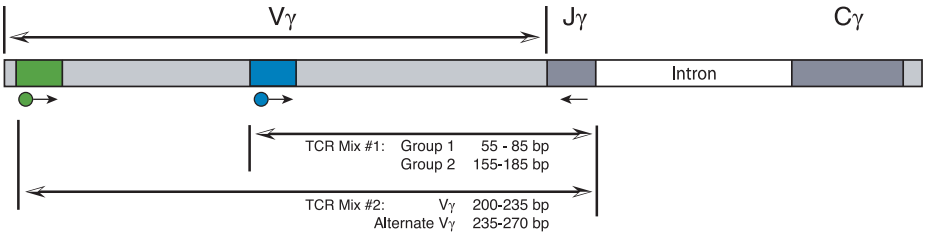


Figure Legend: Simplified figure representing the organization of a rearranged T-cell receptor gamma chain gene on chromosome 7. Colored arrows represent conserved regions within the variable region gene segments targeted by primers. Primers are represented by arrows with the size range of valid products 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.

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

Ordering Information

Catalog #	Products	Quantity
1-207-0051	T-Cell Receptor Gene Rearrangement Assay - ABI Fluorescence Detection	30 reactions

This product is for Research Use Only (RUO).
Not intended for diagnostic purposes.

TCRD Gene Clonality Assays

Assay Use

T-Cell receptor delta (*TCRD*) gene clonality assays are useful for the identification of T-cell clonality, studying clonal T-cell populations, and 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].

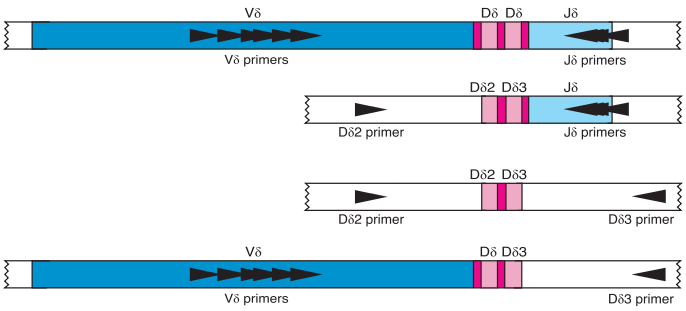
Specimen Requirements

This assay tests extracted and purified genomic DNA (gDNA).

References

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.



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

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.

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

Ordering Information

Catalog #	Products	Quantity
1-206-0010	*TCRD* Gene Clonality Assay - Gel Detection	33 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

These products are for Research Use Only (RUO).
Not intended for diagnostic purposes.

BCL1/J_H Translocation Assay

Assay Use

These assays identify *BCL1*/J_H t(11;14) translocations and are useful for the 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*/J_H Master Mix targets the major translocation cluster (MTC) of the *CCND1* locus (formerly known as *BCL1*) and the joining region (J_H) 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

This aberrant *BCL1*/J_H t(11;14) translocation juxtaposes genes of the immunoglobulin heavy chain (*IGH*) gene on chromosome 14q32 with the cyclin D1 gene on chromosome 11q13. The juxtaposition of *IGH*-sequences results in the transcriptional activation of cyclin D1.[2,3] Cyclin D1 is involved in the regulation of the G1 progression and G1/S transition of the cell cycle.[3] Translocation does not lead to expression of a fusion protein. In fact, oncogenesis is due to a promoter/enhancer exchange, wherein the immunoglobulin gene enhancer stimulates the expression of cyclin D1. Overexpression of cyclin D1, in turn, accelerates passage of transformed cells through the G1 phase.

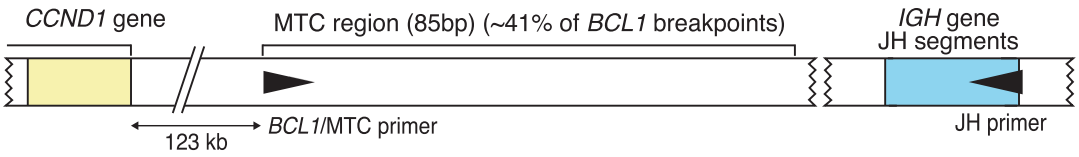
Specimen Requirements

This assay tests extracted and purified genomic DNA (gDNA).

References

1. P Wijers et al., *Leukemia* 17: 2296-2298 (2003).
2. JJM van Dongen et al., *Leukemia* 17: 2257-2317 (2003).
3. Shimazaki C, et. al., (1997). *International Journal of Hematology*. 66(1):111-5.

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



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

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 J_H primer, which are included in the *BCL1*/J_H Master Mix tube.

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/J_H Tube	MTC of *CCND1* + *IGH* J_H	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

Ordering Information

Catalog #	Products	Quantity
1-308-0010	*BCL1*/J_H Translocation Assay - Gel Detection	33 reactions
1-308-0020	*BCL1*/J_H Translocation Assay MegaKit - Gel Detection	330 reactions

These products are for Research Use Only (RUO).
Not intended for diagnostic purposes.

BCL2/J_H t(14;18) Translocation Assays

Assay Use

This Research Use Only assay identifies *BCL2*/J_H t(14;18) translocations.

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. 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

BCL2 translocations are reciprocal chromosome exchanges that place the *bcl*-2 proto-oncogene, located on chromosome 18, under aberrant transcriptional control of the immunoglobulin heavy chain gene, located on chromosome 14. The *bcl*-2 protein is an antagonist to apoptosis (programmed cell death), a normal process designed to eliminate unneeded and damaged cells during hematopoiesis. Increased expression of the *bcl*-2 protein leads to an increase in the levels of B-cells in the body.

Specimen Requirements

This assay tests extracted and purified genomic DNA (gDNA).

References

1. MS Lee et al., *Science* 237: 175-178 (1987).
2. M Crescenzi et al., *Proc. Natl. Acad. Sci. USA* 85: 4869-4873 (1988).

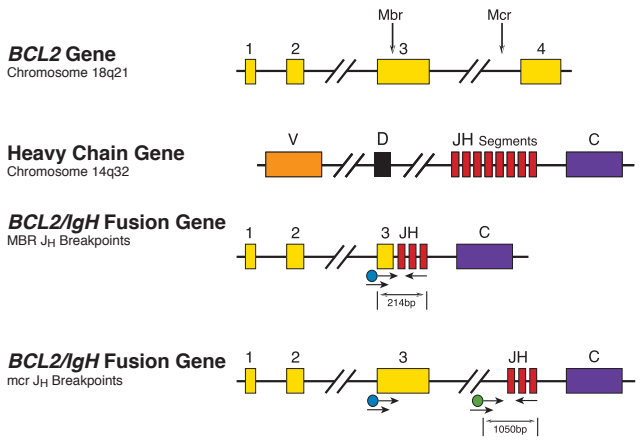


Figure Legend: Simplified view of the genomic organization of the *BCL2* and *IGH* genes on chromosomes 18 and 14, respectively. Yellow boxes represent the exon regions of the *BCL2* gene. Exons of the immunoglobulin heavy chain gene are represented in other colors. The solid black lines represents intron 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.

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

Ordering Information

Catalog #	Products	Quantity
1-309-0010	*BCL2*/JH t(14;18) Translocation Assay - Gel Detection	30 reactions

This product is for Research Use Only (RUO).
Not intended for diagnostic purposes.

BCL2/J_H Translocation Assay

Assay Use

This Research Use Only assay identifies *BCL2*/J_H translocations.

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

BCL2 translocations are reciprocal chromosome exchanges that place the *bcl*-2 proto-oncogene, located on chromosome 18, under aberrant transcriptional control of the immunoglobulin heavy chain gene, located on chromosome 14. The *bcl*-2 protein is an antagonist to apoptosis (programmed cell death), a normal process designed to eliminate unneeded and damaged cells during hematopoiesis. Increased expression of the *bcl*-2 protein leads to an increase in the levels of B-cells in the body.

Specimen Requirements

This assay tests extracted and purified genomic DNA (gDNA).

References

1. PAS Evans et al., *Leukemia* 17: 2298-2301 (2003).
2. JJM van Dongen et al., *Leukemia* 17: 2257-2317 (2003).

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

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

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

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 J_H primer, which are included in the 3 *BCL2*/J_H master mix tubes.

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

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

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

These products are for Research Use Only (RUO).
Not intended for diagnostic purposes.

BCR/ABL t(9;22) Translocation Assays

Assay Use

This Research Use Only assay identifies *BCR/ABL* t(9;22) translocations.

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. 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

BCR/ABL translocations are associated with a variety of hematologic malignancies. The Philadelphia chromosome (Ph1) is a specific chromosomal abnormality that results from reciprocal t(9;22)(q34;ql1) chromosomal rearrangements fuse coding regions of the BCR gene, located on chromosome 22, with the *ABL* receptor-independent tyrosine kinase gene on chromosome 9. This assay detects and identifies the variety of p190-, p210- and p230-type transcripts produced from all known *BCR/ABL* translocations.

Specimen Requirements

This assay tests complementary DNA (cDNA) template.

References

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).

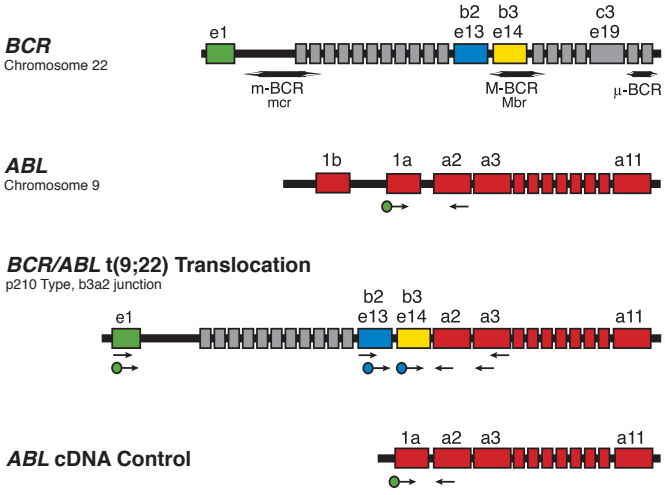


Figure Legend: This figure shows the genomic organization of the *BCR* and *ABL* genes on chromosomes 22 and 9, respectively. Boxes represent exon regions of the *ABL* (red boxes) and *BCR* encoding exons (other colors). The solid black line represents intron regions, which have been left incompletely 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.

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

Ordering Information

Catalog #	Products	Quantity
1-310-0010	*BCR/ABL* t(9;22) Translocation Assay - Gel Detection	30 reactions
1-310-0031	*BCR/ABL* t(9;22) Translocation Assay - ABI Fluorescence Detection	30 reactions

These products are for Research Use Only (RUO).
Not intended for diagnostic purposes.

PML/RARα t(9;22) Translocation Assays

Assay Use

This Research Use Only assay identifies *PML/RARα* t(15;17) translocations.

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 and the variety of Bcr1, Bcr2, and Bcr3 type transcripts expressed from *PML-RARα* translocations. Amplicon products can be analyzed by differential fluorescence detection using capillary electrophoresis. A *PML-RARα* 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

Three *PML/RARα* translocation patterns have been identified in samples with acute myelogenous leukemia (AML): type A is the short (S-form); the breakpoint occurs within breakpoint cluster region 3 (Bcr-3). Type B is the long (L-form); the breakpoint occurs within Bcr-1. There is a third type B variant or variable (V-form); the breakpoint is within Bcr-2. Identification of the *PML/RARα* t(15;17) rearrangements are commonly used in the study of APL because it is correlated with responsiveness to treatment. This RT-PCR method directly identifies the chimeric *PML/RARα* transcripts expressed from all three forms of *PML/RARα* translocations.

Specimen Requirements

This assay tests complementary DNA (cDNA) template.

References

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).

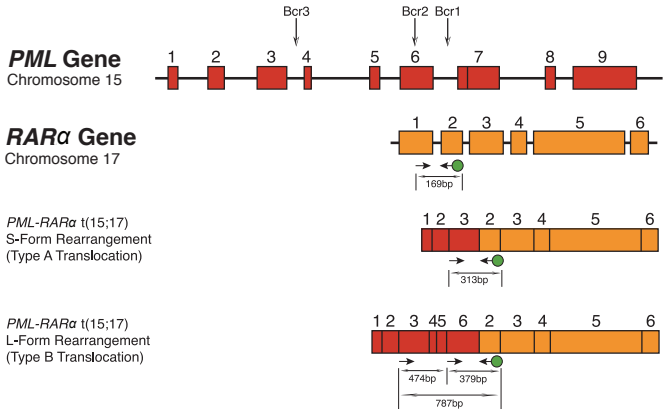


Figure Legend: This figure shows the genomic organization of the PML and RARα genes on chromosomes 15 and 17, respectively. Boxes represent exon regions of the *PML* (red boxes) and *RARα* (orange) encoding exons. The solid black line represents intron regions, which were left incompletely spliced to 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-RARα* translocations are depicted in the lower portion of the figure.

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

Ordering Information

Catalog #	Products	Quantity
1-311-0011	*PML/RARα* t(15;17) Translocation Assay - ABI Fluorescence Detection	30 reactions

This product is for Research Use Only (RUO).
Not intended for diagnostic purposes.

IGH Somatic HyperMutation Assays v2.0

Assay Use

The Research Use Only *IGH* Somatic Hypermutation Assay v2.0 is used to identify clonal rearrangements of the immunoglobulin heavy (*IGH*) chain gene and determine the somatic mutation status of the variable (V) gene sequence and is useful for the study of:

1. Identifying clonal rearrangements of the *IGH* chain gene
2. Assessing the extent of somatic hypermutation in the variable region of the immunoglobulin heavy chain gene
3. 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 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. 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

Rearrangements of the antigen receptor genes occur during ontogeny in B and T lymphocytes. These gene rearrangements are unique in length and sequence for each cell. Therefore, 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] This PCR-based assay employs multiple consensus DNA primers that target conserved genetic regions within the immunoglobulin heavy chain (*IGH*) gene. This test is used to detect and sequence the majority of clonal *IGH* rearrangements from either genomic DNA (gDNA) or complementary DNA (cDNA). Clonal products can be detected using a variety of methods, including gel and capillary electrophoresis. The presence of *IGH* somatic hypermutation (SHM) is defined as greater or equal to 2% difference from the germline variable (V) gene sequence, whereas less than 2% difference is considered evidence of no somatic hypermutation.[3]

Specimen Requirements

This assay tests extracted and purified genomic DNA (gDNA).

References

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).

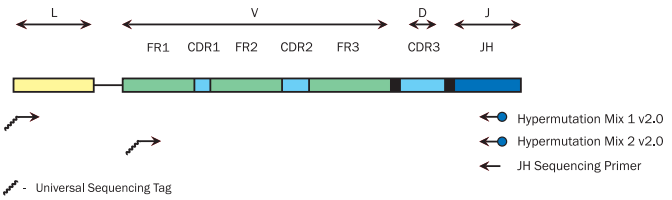


Figure Legend: Simple representation of the organization of a rearranged immunoglobulin heavy chain gene on chromosome 14. Black arrows represent the relative positions of primers that target the conserved Leader (L) and Framework 1 (FR1) regions, and the downstream consensus J_H gene segments.

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 + J_H	1 x 1500 µL tube	10 x 1500 µL tubes
Hypermutation Mix 2 v2.0	Framework 1 + J_H	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 J_H Primer	J_H	1 x 10 µL tube at 100 µM	5 x 10 µL tube

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

These products are for Research Use Only (RUO).
Not intended for diagnostic purposes.

FLT3 Mutation Assays

Assay Use

These Research Use Only assays identify *FLT3* mutations.

Summary and Explanation of the Test

FLT3 Mutation Assays target regions of the *FLT3* gene to identify internal tandem duplication (ITD) mutations and tyrosine kinase domain (TKD) mutations, such as the D835 and I836 mutations. DNA is amplified by PCR, TKD amplicon is enzymatically digested, and *FLT3* mutations are detected via agarose gel (catalog #14120010) or capillary (catalog #14120031) electrophoresis.

Assay kits include three PCR master mixes, along with positive and negative controls. *FLT3* ITD master mix tests for internal tandem duplication mutations. *FLT3* D835 master mix tests for TKD region mutations. 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.

Master mixes contain fluorophore-labeled (capillary) or unlabeled (gel) primer sets as appropriate to kit detection method.

Background

FLT3 is a receptor tyrosine kinase that is normally expressed on many cell types including hematologic stem cells. Mutation of the *FLT3* receptor, by either internal tandem duplication (ITD) of the juxtamembrane domain or point mutation in the activation loop of the tyrosine kinase domain (TKD), causes constitutive activation of the *FLT3* receptor.

Such gain-of-function mutations in the FMS related tyrosine kinase 3 (*FLT3*) gene are the subject of research studies and multiple clinical trials targeting Acute Myeloid Leukemia (AML) subjects. The most prevalent type of *FLT3* mutation is an internal tandem duplication in and around the juxtamembrane domain. The second most common mutation type in the *FLT3* gene is a TKD point mutation in aspartate (D835) or isoleucine (I836).

Specimen Requirements

High quality genomic DNA

References

https://clinicaltrials.gov

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).

Reagents

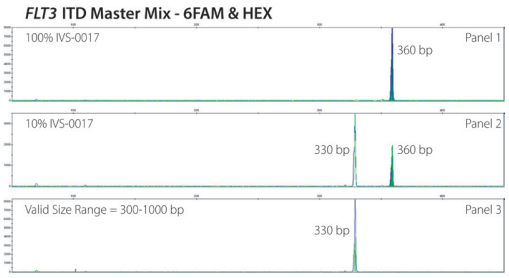
Controls	Concentration	Units in 33 Reaction Assay
IVS-0017 Clonal Control DNA	200 µg/mL	1 x 100 µL tube
IVS-P001 Clonal Control DNA	200 µg/mL	1 x 100 µL tube
IVS-0000 Polyclonal Control DNA	200 µg/mL	1 x 100 µL tube

Master Mixes	Target	Units in 33 Reaction Assay
FLT3 ITD Master Mix	*FLT3* ITD	1 x 1500 µL tube
FLT3 D835 Master Mix	*FLT3* TKD	1 x 1500 µL tube
Specimen Control Size Ladder	Multiple Genes	1 x 1500 µL tube

Gel Electrophoresis Detection

Data was generated using the *FLT3* ITD Master Mix, and amplified products were run on a 2% TBE agarose gel alongside a 100bp DNA size ladder. 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 wild type product.

*IVS-0050 performs comparable to IVS-0017 clonal control DNA, which is included in the kit as the positive control.

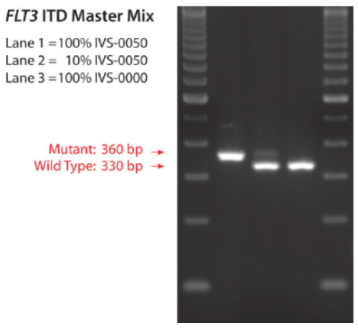


Capillary Electrophoresis Detection (ABI)

Data was generated using the *FLT3* ITD Master Mix and amplified products were run on an ABI 3100 instrument. Templates analyzed follow: Panel 1 is the *FLT3* ITD positive control; panel 2 is a 10% dilution of the positive control; and Panel 3 is IVS-0000, which is representative of a wild type product.

Ordering Information

Catalog #	Products	Quantity
1-412-0010	*FLT3* Mutation Assay - Gel Detection	33 reactions
1-412-0031	*FLT3* Mutation Assay - ABI Fluorescence Detection	33 reactions



These products are for Research Use Only (RUO). Not intended for diagnostic purposes., and additionally not available for sale or use in regions where CE-IVD products are registered.

These products are not available for sale or use in the United States. Please see the LeukoStrat CDx *FLT3* Mutation Assay product pages for IVD product availability in the United States and other global regions.

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*).

LEARN MORE:

invivoscribe.com/products/reagents

Analyte Specific Reagents

Invivoscribe Analyte Specific Reagents (ASRs) target B- and T-cell antigen receptor loci.

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.

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.

Analytical and performance characteristics are not established. ASRs are not available for sale or use outside of the USA.

IGH (Immunoglobulin Heavy Chain Gene Locus)

Description	Catalog #
IGH Framework 1 - 6FAM	A-101-0061
IGH Framework 2 - 6FAM	A-101-0091
IGH Framework 3 - HEX	A-101-0081
IGH FR1 - 6FAM	A-101-0011
IGH FR2 - 6FAM	A-101-0101
IGH FR3 - HEX	A-101-0031
IGH D_H1 - 6 - HEX	A-101-0041
IGH D_H7 - 6FAM	A-101-0051

TRB (T-Cell Receptor Beta Chain Gene Locus)

Description	Catalog #
TCRB V - J1 + 2 - 6FAM & HEX	A-205-0011
TCRB V - J2 - 6FAM	A-205-0021
TCRB D - J1 + 2 - 6FAM & HEX	A-205-0031

IGK (Immunoglobulin Kappa Light Chain Gene Locus)

Description	Catalog #
IGK V - J - 6FAM	A-102-0011
IGK V - K_{de} - 6FAM	A-102-0021

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

Controls, Reagents, and Enzymes

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

LEARN MORE:
invivoscribe.com/products/controls

Introduction

Controls are available in 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.

Chapter Contents

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	◆	◆									
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						◆					

‡These controls can be used as SHM positive controls with ≥2% mutational rates compared to the germline sequence.
€This control does not contain a complete *IGH* V_H-J_H rearrangement and may only be suitable for *IGH* D_H-J_H rearrangements.
*******IGH-CCND1* was previously referred to as *BCL1*/JH.

◆ Gene rearrangement
❍ Partial IGH DH-JH rearrangement
★ Recommended for NGS

Tissue DNA

Standard Concentrations

Our high-quality DNA controls 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

*These controls are general purpose reagents (GPRs).

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

Our high-quality DNA controls are supplied in aliquots of 100 µL and are provided at 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.

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	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

Catalog #	Description
4-088-0190	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

Catalog #	Description
4-088-0370	IVS-0007 Clonal Control 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

Catalog #	Description
4-088-0430	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

Catalog #	Description
4-088-0490	IVS-0009 Clonal Control DNA

These controls are general purpose reagents (GPRs).
‡This control does not contain a complete *IGH* V_H-J_H rearrangement and may only be suitable for *IGH* D_H-J_H rearrangements.

Cell Line 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

Catalog #	Description
4-088-0550	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	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

Catalog #	Description
4-088-1090	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

Catalog #	Description
4-088-1210	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

Catalog #	Description
4-088-1390	IVS-0024 Clonal Control 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

Catalog	Description
4-088-1690	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

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

Catalog	Description
4-088-1750	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

Catalog	Description
4-088-1810	IVS-0031 Clonal Control DNA

These controls are general purpose reagents (GPRs).

LymphoTrack® Low Positive Controls

Measurable 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*
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*

LymphoQuant® 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 *IGH* 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 helps 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*
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*

*LymphoTrack® Low Positive Controls and 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 general purpose reagent (GPR) 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, store the dilutions at -85 °C to -65 °C and minimize the number of freeze-thaw cycles .

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 Clonal Control RNA is negative for BCR-ABL t(9;22) and PML-RARa t(15;17) chromosome translocations.

IVS-0035 may be used as a negative control for other chromosome translocations or diluents for other chromosome translocation positive controls. Please do not hesitate to contact us at support@invivoscribe.com so we can evaluate whether this control may work for your testing needs.

These controls are for Research Use Only (RUO).
Not intended for diagnostic purposes.

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 GPR 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

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.

Catalog #	Description
4-089-1720	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

IVS-0035 Clonal Control RNA

IVS-0035 Clonal Control RNA can be used as a negative control for *BCR-ABL1* t(9;22) and PML-RARa t(15; 17) chromosome translocations.

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

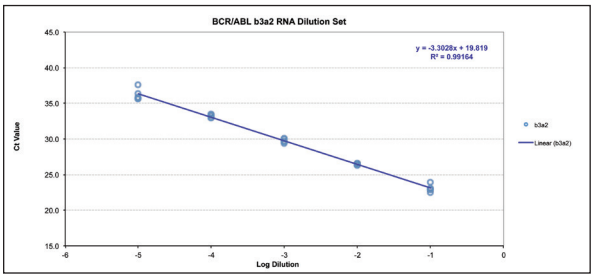
Our *BCR/ABL* b2a2, b3a2, and e1a2 RNA Dilution Sets consist of RNA that has been extracted from *BCR-ABL1* expressing and *BCR-ABL1* negative cell lines. Each set is composed of several dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}) of the *BCR-ABL1* positive RNA diluted (v/v) into RNA purified 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:

1. Routine testing controls for cDNA synthesis, amplification and detection
2. Controls to establish a standard reference curve
3. Proficiency controls
4. Sensitivity controls for specific target assays

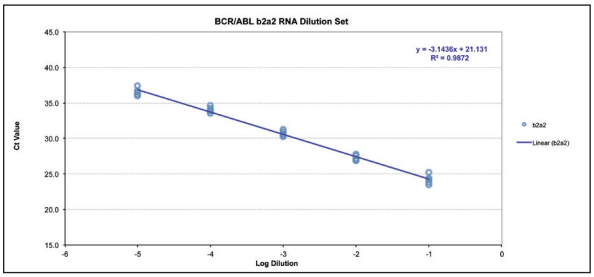
Data

Plot of Ct values (5 replicates) for the 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} dilutions.



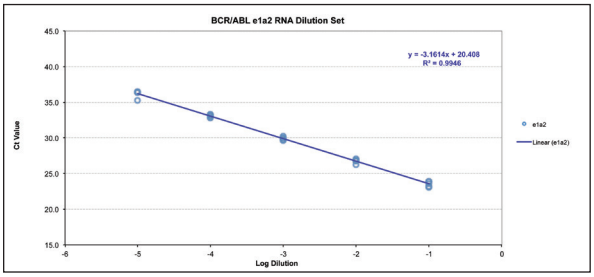
Ordering Information – e14a2 (b3a2)

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 products are for Research Use Only (RUO).
Not intended for diagnostic purposes.

RNA Control Panels

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.

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

These products are for Research Use Only (RUO).
Not intended for diagnostic purposes.

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.

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 base pair 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

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 base pair 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 base pair.

For samples tested on an ABI 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 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.

Catalog #	Description
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

Enzyme

FalconTaq™ DNA Polymerase

FalconTaq™ DNA Polymerase can be used for amplification using PCR to obtain high specificity, sensitivity, and yield. This enzyme has been proven to minimize extension of non-specifically bound primers. Generate reliable results by using FalconTaq™ DNA Polymerase for robust performance.

Catalog #	Description
6-097-0130	FalconTaq™ DNA Polymerase 250 U, 5 U/µL

FalconTaq™ DNA Polymerase is replacing EagleTaq DNA Polymerase, please contact support@invivoscribe.com if you have questions.

These products are for Research Use Only (RUO).
Not intended for diagnostic purposes.

Custom Products

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, and release.

LEARN MORE:
invivoscribe.com/clinical-lab-services

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 email to inquiry@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 and 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 **inquiry@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.

B- or T-cell Clonality Testing Workflow

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.

LEARN MORE:
invivoscribe.com/products/controls

Peripheral blood, Tissue, or FFPE sample

DNA Extraction

PCR Reaction Setup
(Master Mix + Taq* + DNA sample or control)

PCR Amplification

Gel Electrophoresis with Heteroduplex Analysis

STEP 1
Heat denature
94 °C / 5 minutes

STEP 2
Snap chill on ice
1 hour

STEP 3
Load and run
non-denaturing
polyacrylamide gel

STEP 4
Staining

STEP 5
UV illumination

Capillary Electrophoresis

STEP 1
Mix with Hi-Di
Formamide and
size standards

STEP 2
Heat denature
95 °C / 3 minutes

STEP 3
Snap chill on ice
5 minutesl

STEP 4
Load and run
capillary
electrophoresis

STEP 5
Analyze with
fragment analysis
software

Next-Genereation Sequencing

STEP 1
Purify PCR
amplicons

STEP 2
Quantify and
normalize library

STEP 3
Enrich** and
prepare library

STEP 4
Load and run
instrument

STEP 5
Process with
bioinformatics
software

Data Interpretation

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 Ion PGM™ instrument. A flowchart illustrating this workflow is shown below.

*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 bioinformatics software

STEP 7 Analyze results

Notice: The LymphoTrack Dx Assays are *in vitro* diagnostic products and are available in regions that accept CE-IVD products.
*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 bioinformatics software

STEP 7 Analyze results

Notice: The LymphoTrack Dx Assays are *in vitro* diagnostic products and are available in regions that accept CE-IVD products.
*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 measurable 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 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 LymphoTrack 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 LymphoTrack Measurable 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.

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	NOT AVAILABLE

Research Use Only (RUO) Assays	MiSeq™	Ion S5/PGM™
LymphoTrack® *IGHV* Somatic Hypermutation Assays	AVAILABLE	NOT AVAILABLE
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	NOT AVAILABLE

CE-marked assays are *in vitro* diagnostic products and are not available for sale or use within North America.
**Measurable 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, 3500xL, 3500xL Dx. 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, translocation and *FLT3* mutation assays either as RUO, CE-IVD, or IVD.

CE-Marked IVD Assays	Gel	ABI
IdentiClone® *IGH + IGK* B-Cell Clonality Assay	NOT AVAILABLE	AVAILABLE
IdentiClone® *IGH* Gene Clonality Assay	NOT AVAILABLE	AVAILABLE
IdentiClone® *IGK* Gene Clonality Assay	NOT AVAILABLE	AVAILABLE
IdentiClone® *IGL* Gene Clonality Assay	NOT AVAILABLE	AVAILABLE
IdentiClone® *TCRB* Gene Clonality Assay	NOT AVAILABLE	AVAILABLE
IdentiClone® T-Cell Receptor Gamma Gene Rearrangement Assay 2.0	NOT AVAILABLE	AVAILABLE
IdentiClone® *TCRD* Gene Clonality Assay	NOT AVAILABLE	AVAILABLE
LeukoStrat® *FLT3* Mutation Assay 2.0	NOT AVAILABLE	AVAILABLE
LeukoStrat® CDx *FLT3* Mutation Assay (CE-marked)	NOT AVAILABLE	AVAILABLE

IVD Companion Diagnostic Assays	Gel	ABI
LeukoStrat® CDx *FLT3* Mutation Assay IVD (USA)	NOT AVAILABLE	AVAILABLE
LeukoStrat® CDx *FLT3* Mutation Assay (JP)	NOT AVAILABLE	AVAILABLE
LeukoStrat® CDx *FLT3* Mutation Assay (CE-IVD)	NOT AVAILABLE	AVAILABLE

Research Use Only (RUO) Assays	Gel	ABI
FLT3 Mutation Assay	AVAILABLE	AVAILABLE
IGH + IGK B-Cell Clonality Assay	AVAILABLE	AVAILABLE
IGH Gene Rearrangement Assay	NOT AVAILABLE	AVAILABLE
IGH Gene Clonality Assay	AVAILABLE	AVAILABLE
IGH Somatic Hypermutation Assay v2.0	AVAILABLE	AVAILABLE
IGL Gene Clonality Assay	AVAILABLE	AVAILABLE
TCRB Gene Clonality Assay	AVAILABLE	AVAILABLE
T-Cell Receptor Gamma Gene Rearrangement Assay	NOT AVAILABLE	AVAILABLE
T-Cell Receptor Gamma Gene Rearrangement Assay 2.0	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/RARα t(15;17) Translocation Assay	NOT AVAILABLE	AVAILABLE

CE-marked assays are *in vitro* diagnostic products and are available in regions that accept CE-IVD products.
IVD assays are *in vitro* diagnostic products, and are available for sale or use in regions indicated.

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 V_H – J_H 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 available 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 and Windows 10 (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 VH-JH 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 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).

Product List by Catalog Number

Companion Diagnostic Assays
Capillary Analysis

K-412-0291 LeukoStrat® CDx *FLT3* Mutation Assay (CE-IVD)
K-412-0281 LeukoStrat® CDx *FLT3* Software (CE-IVD)
K-412-0331 LeukoStrat® CDx *FLT3* Mutation Assay (JP)
K-412-0341 LeukoStrat® CDx *FLT3* Software (JP)
K-412-0361 LeukoStrat® CDx *FLT3* Mutation Assay (USA)
K-412-0371 LeukoStrat® CDx *FLT3* Software (USA)

Research Use Only Assays
Capillary & Gel Fragment Analysis

1-100-0010 *IGH + IGK* B-Cell Clonality Assay – 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
1-101-0020 *IGH* Gene Clonality Assay – 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
1-102-0031 *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
1-103-0021 *IGL* Gene 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
1-206-0021 *TCRD* Gene Clonality Assay MegaKit – ABI Fluorescence Detection
1-207-0051 T-Cell Receptor Gamma Gene Rearrangement Assay – 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
1-309-0040 *BCL2/J*H Translocation Assay MegaKit – Gel Detection
1-310-0010 *BCR/ABL* t(9;22) Translocation Assay – Gel Detection
1-310-0031 *BCR/ABL* t(9;22) Translocation Assay – ABI Fluorescence Detection
1-311-0011 *PML/RARa* t(15;17) Translocation Assay – ABI Fluorescence Detection
1-412-0010 *FLT3* Mutation Assay - Gel Detection
1-412-0031 *FLT3* Mutation Assay - ABI Fluorescence Detection

Master Mixes

2-096-0020 Specimen Control Size Ladder – Unlabeled
2-096-0021 Specimen Control Size Ladder – 6FAM
2-101-0011 *IGH* Tube A – 6FAM
2-101-0031 *IGH* Tube C – HEX
2-101-0041 *IGH* Tube D – HEX
2-101-0051 *IGH* Tube E – 6FAM
2-101-0061 *IGH* Framework 1 (FR1) – 6FAM
2-101-0081 *IGH* Framework 3 (FR3) – HEX
2-101-0091 *IGH* Framework 2 (FR2) – 6FAM
2-101-0101 *IGH* Tube B – 6FAM
2-101-0180 Hypermutation Mix 2 v2.0 – Unlabeled
2-102-0011 *IGK* Tube A – 6FAM
2-102-0021 *IGK* Tube B – 6FAM
2-103-0011 *IGL* Tube – 6FAM
2-205-0011 *TCRB* Tube A – 6FAM & HEX
2-205-0021 *TCRB* Tube B – 6FAM
2-205-0031 *TCRB* Tube C – 6FAM & HEX
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
2-207-0091 *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
2-310-0040 *BCR/ABL* t(9;22) Mix 1b – Unlabeled
2-310-0050 *BCR/ABL* t(9;22) Mix 2b – Unlabeled
2-310-0060 *BCR/ABL* t(9;22) Mix 2c – Unlabeled
2-310-0070 *BCR/ABL* t(9;22) Mix 3b – Unlabeled
2-310-0080 *BCR/ABL* t(9;22) Mix 3c – Unlabeled
2-310-0090 *BCR/ABL* t(9;22) Mix 3d – Unlabeled
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

Next-Generation Sequencing
RUO LymphoTrack Assays

7-121-0059 LymphoTrack® *IGHV* Somatic Hypermutation Assay Kit A – MiSeq™
7-121-0069 LymphoTrack® *IGHV* Somatic Hypermutation Assay Panel – MiSeq™
7-121-0129 LymphoTrack® *IGH* FR1/2/3 Assay Kit A – MiSeq™
7-121-0139 LymphoTrack® *IGH* FR1/2/3 Assay Panel – MiSeq™
7-121-0009 LymphoTrack® *IGH* FR1 Assay Kit A – MiSeq™
7-121-0039 LymphoTrack® *IGH* FR1 Assay Panel – MiSeq™
7-121-0089 LymphoTrack® *IGH* FR2 Assay Kit A – MiSeq™
7-121-0099 LymphoTrack® *IGH* FR2 Assay Panel – MiSeq™
7-121-0109 LymphoTrack® *IGH* FR3 Assay Kit A – MiSeq™
7-121-0119 LymphoTrack® *IGH* FR3 Assay Panel – MiSeq™
7-121-0057 LymphoTrack® *IGH* FR1/2/3 Assay – S5/PGM™
7-121-0007 LymphoTrack® *IGH* FR1 Assay – S5/PGM™
7-121-0037 LymphoTrack® *IGH* FR2 Assay – S5/PGM™
7-121-0047 LymphoTrack® *IGH* FR3 Assay – S5/PGM™
7-122-0009 LymphoTrack® *IGK* Assay Kit A – MiSeq™
7-122-0019 LymphoTrack® *IGK* Assay Panel – MiSeq™
7-122-0007 LymphoTrack® *IGK* Assay – S5/PGM™
7-225-0009 LymphoTrack® *TRB* Assay Kit A – MiSeq™
7-225-0019 LymphoTrack® *TRB* Assay Panel – MiSeq™
7-227-0019 LymphoTrack® *TRG* Assay Kit A – MiSeq™
7-227-0009 LymphoTrack® *TRG* Assay Panel – MiSeq™
7-227-0007 LymphoTrack® *TRG* Assay – S5/PGM™
7-500-0007 LymphoTrack® Software – S5/PGM™
7-500-0008 LymphoTrack® MRD Software
7-500-0009 LymphoTrack® Software – MiSeq™

Capillary Fragment Analysis CE-IVD IdentiClone® Assays

9-100-0031 IdentiClone® *IGH + IGK* B-Cell Clonality Assay – ABI Fluorescence Detection
9-100-0041 IdentiClone® *IGH + IGK* B-Cell Clonality Assay MegaKit – ABI Fluorescence Detection
9-101-0061 IdentiClone® *IGH* Gene Clonality Assay – ABI Fluorescence Detection
9-101-0081 IdentiClone® *IGH* Gene Clonality Assay MegaKit – ABI Fluorescence Detection
9-102-0021 IdentiClone® *IGK* Gene Clonality Assay – ABI Fluorescence Detection
9-102-0031 IdentiClone® *IGK* Gene Clonality Assay MegaKit – ABI Fluorescence Detection
9-103-0011 IdentiClone® *IGL* Gene Clonality Assay – ABI Fluorescence Detection
9-205-0011 IdentiClone® *TCRB* Gene Clonality Assay – ABI Fluorescence Detection
9-205-0021 IdentiClone® *TCRB* Gene Clonality Assay MegaKit – ABI Fluorescence Detection
9-206-0011 IdentiClone® *TCRD* Gene Clonality Assay – ABI Fluorescence Detection
9-206-0021 IdentiClone® *TCRD* Gene Clonality Assay MegaKit – ABI Fluorescence Detection
9-207-0101 IdentiClone® T-Cell Receptor Gamma Gene Rearrangement Assay 2.0 – ABI Fluorescence Detection
9-207-0111 IdentiClone® T-Cell Receptor Gamma Gene Rearrangement Assay 2.0 MegaKit – ABI Fluorescence Detection

Capillary Fragment Analysis CE-IVD LeukoStrat® Assays

9-412-0091 LeukoStrat® *FLT3* Mutation Assay 2.0 – ABI Fluorescence Detection
K-412-0291 LeukoStrat® CDx *FLT3* Mutation Assay 33 reactions – ABI Fluorescence Detection
K-412-0281 LeukoStrat® CDx *FLT3* Mutation Assay Software

Analyte Specific Reagents

A-412-0071 *FLT3* ITD MM – 6FAM & HEX – ASR
A-412-0081 *FLT3* TKD MM – 6FAM – ASR
A-101-0011 *IGH* FR1 – 6FAM
A-101-0020 *IGH* FR2 – Unlabeled
A-101-0030 *IGH* FR3 – Unlabeled
A-101-0031 *IGH* FR3 – HEX
A-101-0041 *IGH* DH1 - 6 – HEX
A-101-0051 *IGH* DH7 – 6FAM
A-101-0061 *IGH* Framework 1 – 6FAM
A-101-0070 *IGH* Framework 2 – Unlabeled
A-101-0080 *IGH* Framework 3 – Unlabeled
A-101-0081 *IGH* Framework 3 – HEX
A-101-0091 *IGH* Framework 2 – 6FAM
A-101-0101 *IGH* FR2 – 6FAM
A-102-0010 *IGK* V - J – Unlabeled
A-102-0011 *IGK* V - J – 6FAM
A-102-0020 *IGK* V - K_{de} – Unlabeled
A-102-0021 *IGK* V - K_{de} – 6FAM
A-103-0011 *IGL* V - J – 6FAM
A-205-0010 *TCRB* V - J1+2 – Unlabeled
A-205-0011 *TCRB* V - J1+2 – 6FAM & HEX
A-205-0020 *TCRB* V - J2 – Unlabeled
A-205-0021 *TCRB* V - J2 – 6FAM
A-205-0031 *TCRB* D - J1+ 2 – 6FAM & HEX
A-207-0021 *TCRG* V(1-8)J – HEX
A-207-0071 *TCRG* V(1-8,9)J – 6FAM
A-207-0091 *TCRG* V(2-5,8-11)J1+2+P – 6FAM
A-309-0050 *BCL2/*J_H Mbr – Unlabeled
A-309-0060 *BCL2/*J_H 3'Mbr – Unlabeled
A-309-0070 *BCL2/*J_H mcr – Unlabeled

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

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

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
4-088-0370 IVS-0007 Clonal Control DNA
4-088-0430 IVS-0008 Clonal Control DNA
4-088-0490 IVS-0009 Clonal Control DNA
4-088-0550 IVS-0010 Clonal Control DNA
4-088-0730 IVS-0013 Clonal Control DNA
4-088-1090 IVS-0019 Clonal Control DNA
4-088-1210 IVS-0021 Clonal Control DNA
4-088-1390 IVS-0024 Clonal Control DNA
4-088-1690 IVS-0029 Clonal Control DNA
4-088-1750 IVS-0030 Clonal Control DNA
4-088-1810 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
4-089-0910 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
4-089-2800 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
4-089-3070 IVS-0035 Clonal Control RNA

Tissue DNA Control

4-092-0010 IVS-0000 Polyclonal Control DNA

LymphoTrack® Low Positive Controls

4-088-0098 LymphoTrack® B-cell Low Positive Control
4-088-0108 LymphoTrack® T-cell Low Positive Control

LymphoQuant® Internal Controls

4-088-0118 LymphoQuant® B-cell Internal Control
4-088-0128 LymphoQuant® T-cell Internal Control

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

6-098-0061 HI-Deionized Formamide with ROX Size Standard (ABI 3100)

Taq DNA Polymerases

6-097-0130 FalconTaq DNA Polymerase

Next-Generation Sequencing CE-IVD LymphoTrack® Dx Assays

9-121-0007 LymphoTrack® Dx *IGH* FR1 Assay – S5/PGM™
9-121-0009 LymphoTrack® Dx *IGH* FR1 Assay Kit A – MiSeq™
9-121-0037 LymphoTrack® Dx *IGH* FR2 Assay – S5/PGM™
9-121-0039 LymphoTrack® Dx *IGH* FR1 Assay Panel – MiSeq™
9-121-0047 LymphoTrack® Dx *IGH* FR3 Assay – S5/PGM™
9-121-0057 LymphoTrack® Dx *IGH* FR1/2/3 Assay – S5/PGM™
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™
9-121-0089 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™
9-121-0129 LymphoTrack® Dx *IGH* FR1/2/3 Assay Kit A – MiSeq™
9-121-0139 LymphoTrack® Dx *IGH* FR1/2/3 Assay Panel – MiSeq™
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™
9-227-0007 LymphoTrack® Dx *TRG* Assay – S5/PGM™
9-227-0009 LymphoTrack® Dx *TRG* Assay Panel – MiSeq™
9-227-0019 LymphoTrack® Dx *TRG* Assay Kit A – MiSeq™
9-500-0007 LymphoTrack® Dx Software – S5/PGM™
9-500-0009 LymphoTrack® Dx Software – MiSeq™

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Intended Uses

Research Use Only products have not been approved by any regulatory agency for clinical use and are not for diagnostic or therapeutic use. Our CE-marked assays, sold in Europe and not for sale or use in the United States, are *in vitro* diagnostic devices intended for detection of clonal gene rearrangements and translocations for patients with suspected lymphoproliferations.

Patent Notice

Many of the products described herein are covered by one or more patents including European Patent No. 1549764, European Patent No. 2418287, European Patent No. 2460889, European Patent No. 1633884, Japanese Patent No. 4708029, United States Patent No. 7,785,783, United States Patent No. 8859748 and United States Patent No. 10280462, Japanese Patent No. 6189600, Brazilian Patent No. PI0410283, Canadian Patent No. 2525122, Indian Patent No. 243620, Mexican Patent No. 286493, Chinese Patent No. 1806051, and Korean Patent No. 10-1215194. All of these patents are licensed to Invivoscribe Inc.

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