IdentiClone®

Instructions for Use

CE IVD

IdentiClone® *TCRB* + *TCRG* T-Cell Clonality Assay

For identification of clonal T cell receptor beta chain and gamma chain gene rearrangements.

IVD For *In Vitro* Diagnostic Use

invivoscribe

Storage Conditions: **-85ºC to -65ºC**
(DNA controls may be separated from assay kits and stored at 2°C to 8°C)

Catalog#	Products	Quantity
REF 92000010	IdentiClone *TCRB* + *TCRG* T-Cell Clonality Assay – Gel Detection	33 Reactions

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1. Intended Use

The IdentiClone *TCRB* + *TCRG* T-Cell Clonality Assay is an *in vitro* diagnostic product intended for PCR-based detection of clonal T-cell receptor beta chain and gamma chain gene rearrangements in patients with suspect lymphoproliferations. Specifically, the *TCRB* + *TCRG* T-Cell 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

2. Summary and Explanation of the Test

2.1. Background

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 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 IdentiClone PCR assay employs multiple consensus DNA primers that target conserved genetic regions within the T cell receptor beta chain and gamma chain genes. This test is used to detect the vast majority of clonal T-cell malignancies from DNA. Test products can be analyzed using a variety of detection formats, including gel and capillary electrophoresis.

Gene rearrangement analysis can also be performed by Southern Blot (SB)-based techniques. Although SB analysis is very reliable, it is increasingly replaced by PCR techniques because of the greater efficiency and sensitivity of PCR. Moreover, PCR is relatively easy, less labor intensive and requires much lower quantities of high molecular weight DNA than SB tests. In addition, PCR can often be performed on DNA isolated from paraffin-embedded tissue, whereas SB cannot be performed because the DNA is often degraded. Therefore there is a strong need to replace SB analysis with reliable PCR techniques.

2.2. Summary

IdentiClone assays represent a new approach to PCR-based clonality testing. These standardized assays were carefully optimized testing positive and negative control samples using multiplex master mixes. Assay development was followed by extensive validation including the testing of more than 400 clinical samples using Revised European/American Lymphoma (REAL) Classification. Testing was done at more than thirty prominent independent testing centers throughout Europe in a collaborative study known as the BIOMED-2 Concerted Action. Results from this BIOMED-2 study appear in *Leukemia*, a leading peer-reviewed journal.[2] In a Leukemia 2007 article, testing for both *TCRB* and *TCRG* gene rearrangements led to 94% sensitivity, compared to 91% for *TCRB* and 89% for *TCRG* when tested alone. It may also increase the reliability of the tests as it's more likely that the clonal products will be detected in more than one tube.[4]

The gel detection based assays cannot reliably detect clonal populations comprising less than 5% of the total lymphocyte cell population. Always interpret the results of molecular clonality tests in the context of clinical, histological and immunophenotypic data.

This test kit includes 6 master mixes. *TCRB* Tubes A and B target framework regions within the variable region and the joining region of the *TCR* beta chain locus. *TCRB* Tube C targets the diversity and joining regions of the *TCR* beta chain locus. *TCRG* Tube A contains primers that target the Vγ1-8 + Vγ10 genes and Jγ1.1, Jγ1.3, Jγ2.1 and Jγ2.3 genes (also known as JγP1, Jγ1, JγP2 and Jγ2 respectively). *TCRG* Tube B contains primers that target the Vγ9 + Vγ11 genes and Jγ1.1, Jγ1.3, Jγ2.1 and Jγ2.3 genes. Lastly, the Specimen Control Size Ladder master mix, targets multiple genes and generates a series of amplicons of approximately 100, 200, 300, 400, and 600 base pairs to ensure that the quality and quantity of input DNA is adequate to yield a valid result. A single thermal cycler program and similar detection methodologies are used with all of our Gene Clonality Assays. This improves consistency and facilitates cross training on a broad range of different assays.

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

3. Principles of the Procedure

3.1. Polymerase Chain Reaction (PCR)

PCR assays are routinely used for the identification of clonal T-cell populations. These tests amplify the DNA between primers that target the conserved variable (V) regions and the conserved joining (J) regions (*TCRB* Tubes A & B and *TCRG* Tubes A & B), as well as the diversity (D) and joining regions (*TCRB* Tube C). These conserved regions lie on either side of an area within the V-J region where programmed genetic rearrangements occur during maturation of all B and T lymphocytes. The antigen receptor genes that undergo rearrangement are the immunoglobulin heavy chain and light chains in B-cells and the T cell receptor genes in T-cells. Each B- and T-cell has a single productive V-J rearrangement that is unique in both length and sequence. Therefore, when DNA from a normal or polyclonal population is amplified using DNA primers that flank the V-J region, a bell-shaped curve (Gaussian distribution) of amplicon products within an expected size range is produced. On a gel, this distribution of products is seen as a smear. This Gaussian distribution reflects the heterogeneous population of V-J rearrangements. (In certain cases, where lymphocyte DNA is not present, no product is seen.) For DNA from samples containing a clonal population, the yield is one or two prominent amplified products (amplicons) within a diminished polyclonal background.

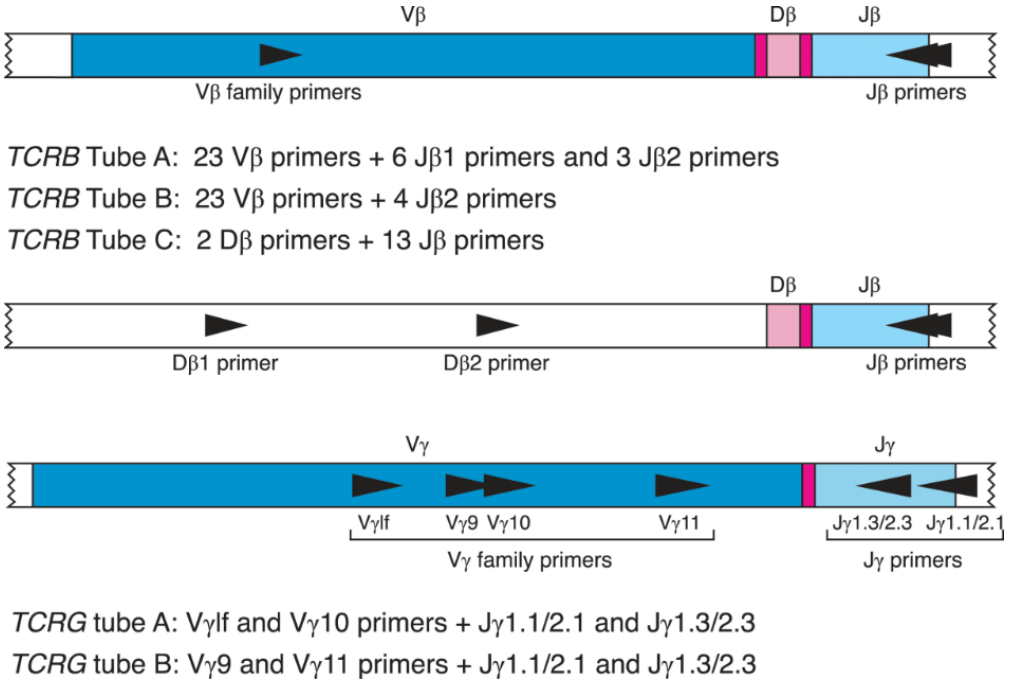

Figure 1. This is a simplified diagram of a representative rearranged T cell receptor beta gene and the T cell receptor gamma gene showing the approximate placement of the upstream and downstream DNA primers. The numbers of primers and their specificity are listed for master mix *TCRB* Tubes A, B and C and *TCRG* Tubes A and B. (The Vγ1f primer is a consensus primer that targets Vγ1 through Vγ8).

Since the antigen receptor genes are polymorphic (consisting of a heterogeneous population of related DNA sequences), it is difficult to employ a single set of DNA primer sequences to target all of the conserved flanking regions around the V-J rearrangement. N-region diversity and somatic mutation further scramble the DNA sequences in these regions. Therefore, multiplex master mixes, which target several FR regions, are required to identify the majority of clonal rearrangements. As indicated, clonal rearrangements are identified as prominent, single-sized products within the background of different-sized amplicon products that form a Gaussian distribution around a statistically favored, average-sized rearrangement.

3.2. Gel Detection

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

A heteroduplex analysis can also be performed and run on a polyacrylamide gel to differentiate clonal and non-clonal PCR products. A heteroduplex analysis involves denaturing the PCR products at a high temperature, then quickly re-annealing the DNA strands by suddenly reducing the temperature. This causes a large portion of DNA strands to incorrectly bind to other non-homologous strands creating loops in the DNA. These loops cause a significant reduction in the ability of the DNA to migrate through a polyacrylamide gel. However, if the majority of the PCR products are clonal, when a heteroduplex analysis is performed, most of these PCR products will correctly re-anneal with a homologous strand. These PCR products will run normally through the polyacrylamide gel. Therefore, in a clonal sample with a polyclonal background, a heteroduplex analysis will cause most of the polyclonal product to run much slower through the polyacrylamide gel, thereby increasing their separation and the ability to identify the clonal band(s).

4. Reagents

4.1. Reagent Components

Table 1. Available Kits

Catalog Number	Products	Quantity
REF 92000010	IdentiClone *TCRB* + *TCRG* T-Cell Clonality Assay – Gel Detection	33 Reactions

Reagent	Catalog #	Reagent Components (active ingredients)	Unit Quantity	92000010 # of Units	Storage Temp.
Master Mixes	22050010CE	***TCRB* Tube A - Unlabeled** Multiple oligonucleotides targeting the Vβ + Jβ1 + Jβ2 regions of the T cell receptor beta gene in a buffered salt solution.	1500 µL	1	-85°C to -65°C
	22050020CE	***TCRB* Tube B - Unlabeled** Multiple oligonucleotides targeting the Vβ + Jβ2 regions of the T cell receptor beta gene in a buffered salt solution.	1500 µL	1	
	22050030CE	***TCRB* Tube C - Unlabeled** Multiple oligonucleotides targeting the Dβ + Jβ1 + Jβ2 regions of the T cell receptor beta gene in a buffered salt solution.	1500 µL	1	
	22070030CE	***TCRG* Tube A – Unlabeled** Multiple oligonucleotides targeting the Vγ1-8, Vγ10 + multiple Jγ regions of the T cell receptor gamma gene in a buffered salt solution.	1500 µL	1	
	22070040CE	***TCRG* Tube B – Unlabeled** Multiple oligonucleotides targeting the Vγ9, Vγ11+ multiple Jγ regions of the T cell receptor gamma gene in a buffered salt solution.	1500 µL	1	
Template Amplification Control Master Mix	20960020	**Specimen Control Size Ladder - Unlabeled** Multiple oligonucleotides targeting housekeeping genes.	1500 µL	1	
Positive Control DNA	40881210	**IVS-0021 Clonal Control DNA** 200 µg/mL of DNA in 1/10th TE solution	100 µL	1	2°C to 8°C or -85°C to -65°C
	40880490	**IVS-0009 Clonal Control DNA** 200 µg/mL of DNA in 1/10th TE solution	100 µL	1	
	40880190	**IVS-0004 Clonal Control DNA** 200 µg/mL of DNA in 1/10th TE solution	100 µL	1	
Negative (Normal) Control DNA	40920010	**IVS-0000 Polyclonal Control DNA** 200 µg/mL of DNA in 1/10th TE solution	100 µL	1	

4.2. Warnings and Precautions

- **IVD This product is For *In Vitro* Diagnostic Use**
- Use this assay kit as a system. Do not substitute other manufacturer's reagents. Dilution, reducing amplification reaction volumes or other deviation in this protocol may affect the performance of this test and/or nullify any limited sublicense that comes with the purchase of this testing kit.
- Materials are stable until the labeled expiration date when stored and handled as directed. Do not use kits beyond their expiration date.
- Close adherence to the protocol will assure optimal performance and reproducibility. Ensure correct thermal cycler program is used, as other programs may provide inaccurate/faulty data, such as false positive and false negative results.
- Do not mix or combine reagents from kits with different lot numbers.
- Laboratory personnel are reminded to wear appropriate personal protective equipment and follow good laboratory practices and universal precautions when working with specimens. Handle specimens in approved biological safety containment facilities and open only in certified biological safety cabinets. Use molecular biology grade water for the preparation of specimen DNA.
- Due to the analytical sensitivity of this test, use extreme care to avoid the contamination of reagents or amplification mixtures with samples, controls or amplified materials. Closely monitor all reagents for signs of contamination (e.g., negative controls giving positive signals). Discard reagents suspected of contamination.
- To minimize contamination, wear clean gloves when handling samples and reagents and routinely clean work areas and pipettes prior to doing PCR.
- Autoclaving does not eliminate DNA contamination. Always follow uni-directional workflow between separate areas in the PCR laboratory: begin with Master Mix preparation, move to specimen preparation, then to amplification and finally to detection. Do not bring amplified DNA into the areas designated for master mix or specimen preparation.
- Dedicate all pipettes, pipette tips and any equipment used in a particular area to that area of the laboratory.
- Use sterile, disposable plastic ware whenever possible to avoid RNase, DNase or cross-contamination.

4.3. Storage and Handling

- **Store assay kits at -85°C to -65°C** until ready to use.
- The optimum storage temperature for DNA controls is 2°C to 8°C, but DNA controls can be stored at -85°C to -65°C.
- All reagents and controls must be thawed and vortexed or mixed thoroughly prior to use to ensure that they are resuspended completely. Excessive vortexing may shear DNA and cause labeled primers to lose their fluorophors.
- Materials are stable until the labeled expiration date when stored and handled as directed. Do not use kits beyond their expiration date.
- Due to high salt concentrations, PCR master mixes are sensitive to freeze/thaw cycles. Aliquot master mixes into sterile o-ring screw-cap tubes if necessary.

5. Instruments

5.1. Thermal cycler

- Use or Function: Amplification of DNA samples
- Suggested Instrument: Veriti™ thermal cycler or equivalent
- Performance characteristics and specification:
  - Minimum Thermal Range: 15°C to 96°C
  - Minimum Ramping Speed: 0.8°C/sec
- Follow manufacturer's installation, operation, calibration and maintenance procedures.
- See section 7.4 *Amplification* for thermal cycler program.

5.2. Electrophoresis Unit

- Use or Function: DNA fragment separation
- Performance Characteristics and Specification:
  - Capable of running at 35V to 135V for extended times
- Follow manufacturer's installation, operation, calibration and maintenance procedures.

5.3. UV Illumination Unit

- Use or Function: DNA detection
- Performance Characteristics and Specification:
  - Capable of emitting light at a wavelength of ~302 nm
- Follow manufacturer's installation, operation, calibration and maintenance procedures.

6. Specimen Collection and Preparation

6.1. Precautions

Biological specimens from humans may contain potentially infectious materials. Handle all specimens in accordance with the OSHA Standard on Bloodborne Pathogens or Biosafety Level 2.

6.2. Interfering Substances

The following substances are known to interfere with PCR:

- Divalent cation chelators
- Low retention pipette tips
- EDTA (not significant at low concentrations)
- Heparin

6.3. Specimen Requirements and Handling

This assay tests **genomic DNA** from the following sources:

- 5 cc of peripheral blood, bone marrow biopsy or bone marrow aspirate anti-coagulated with heparin or EDTA (stored at 2°C to 8°C and shipped at ambient temperature)
- Minimum 5 mm cube of tissue (stored and shipped frozen; or stored and shipped in RPMI 1640 at ambient temperature or on ice)
- 3 µg of genomic DNA (stored at 2°C to 8°C and shipped at ambient temperature)
- Formalin-fixed paraffin embedded tissue or slides (stored and shipped at ambient temperature)

6.4. Sample Preparation

Extract the genomic DNA from patient specimens as soon as possible. Resuspend DNA to a final concentration of 100 µg to 400 µg per mL in $1/10^{th}$ TE (1 mM Tris-HCl, pH 8.0; 0.1 mM EDTA) or in molecular biology grade or USP water. This is a robust assay system. A wide range of DNA concentrations will generate a valid result. Therefore, quantifying and adjusting DNA concentrations is generally not necessary. Testing sample DNA with the Specimen Control Size Ladder master mix will ensure that DNA of sufficient quality and quantity was present to yield a valid result.

6.5. Sample Storage

Store genomic DNA at 2°C to 8°C or at -85°C to -65°C until use.

7. Assay Procedure

7.1. Materials Provided

Table 2. Materials Provided

Catalog #	Description
REF 22050010CE	*TCRB* Tube A – Unlabeled
REF 22050020CE	*TCRB* Tube B – Unlabeled
REF 22050030CE	*TCRB* Tube C – Unlabeled
REF 22070030CE	*TCRG* Tube A – Unlabeled
REF 22070040CE	*TCRG* Tube B – Unlabeled
REF 20960020	Specimen Control Size Ladder – Unlabeled
REF 40881210	IVS-0021 Clonal Control DNA
REF 40880490	IVS-0009 Clonal Control DNA
REF 40880190	IVS-0004 Clonal Control DNA
REF 40920010	IVS-0000 Polyclonal Control DNA

7.2. Materials Required (not provided)

Table 3. Materials Required (not provided)

Reagent/Material	Recommended Reagents/Materials and Suppliers	Catalog #	Notes
DNA Polymerase	Roche: • EagleTaq DNA Polymerase Invivoscribe: • EagleTaq DNA Polymerase[1] or equivalent	05206944190 60970100	N/A
Glass Distilled De-ionized Molecular Biology Grade or USP Water	N/A	N/A	Sterile and free of DNase and RNase.
Calibrated Pipettes	Rainin: • P-2, P-20, P-200 and P-1000 pipettes • Or SL-2, SL-20, SL-200 and SL-1000 pipettes	N/A	Must be able to accurately measure volumes between 1 µL and 1000 µL.
Thermal cycler	Thermo Fisher Scientific: • Veriti Dx Thermal Cycler Bio-Rad: • PTC-100 or PTC-200, PTC-220, PTC-240 Perkin-Elmer • PE 9600 or PE 9700	N/A	N/A
Vortex Mixer	N/A	N/A	N/A
PCR plates or tubes	N/A	N/A	Sterile
Filter barrier pipette tips	N/A	N/A	Sterile, RNase/DNase/Pyrogen-free
Microcentrifuge tubes	N/A	N/A	Sterile
Gel Electrophoresis Unit	N/A	N/A	For polyacrylamide gels
Ethidium Bromide	Thermo Fisher Scientific: • UltraPure™ 10 mg/mL Ethidium Bromide	15585-011	N/A

Table 3. Materials Required (not provided)

Reagent/Material	Recommended Reagents/Materials and Suppliers	Catalog #	Notes
6% Polyacrylamide Gels	Thermo Fisher Scientific: • Novex® TBE Gels (6%, 12 well)	EC62652Box	N/A
TBE Running Buffer	Thermo Fisher Scientific: • Novex® TBE Running Buffer (5X)	LC6675	Dilute 1:5 prior to use.
Gel Loading Buffer	Thermo Fisher Scientific: • 10X BlueJuice™ Gel Loading Buffer • Novex® Hi-Density TBE Sample Buffer (5X)	10816-015 LC6678	N/A
100 bp DNA Ladder	Thermo Fisher Scientific: • TrackIt™ 100 bp DNA Ladder	10488-058	N/A

[1]**Note:** This product is for sale and use in the European Economic Area only. It is not to be resold or transferred to another party. See also Legal Notice in section 15.

7.3. Reagent Preparation

- Test all unknown samples using the Specimen Control Size Ladder master mix. This is to ensure that no inhibitors of amplification are present and there is DNA of sufficient quality and quantity to generate a valid result.
- Singlicate test results are valid; however, we recommend **duplicate** testing when possible. If duplicate testing provides inconsistent results, re-testing or re-evaluation of the sample is necessary.
- **Test positive**, **negative** and **no template** controls for each of the master mixes.
- It is recommended to batch multiple samples in a run to avoid running out of the negative control (IVS-0000 Polyclonal Control DNA). If batching samples is not practical in your laboratory setting, IVS-0000 Polyclonal Control DNA is also available for purchase separately.

7.3.1. Using gloved hands, remove the master mixes from the freezer. Allow the tubes to thaw completely; then gently vortex to mix.

7.3.2. In containment hood or dead air box, remove an appropriate aliquot from each master mix to individual clean, sterile microcentrifuge tubes.

- Aliquot volumes are 45 µL for each reaction.
- Add an additional reaction for every 15 reactions to correct for pipetting errors. Thus, for each master mix (except for the Specimen Control Size Ladder), the number of reactions (**n**) is:

n = 2 × # of samples	(run each sample in duplicate)
+ 1	positive control DNA (See Table 6)
+ 1	negative control DNA (IVS-0000 Polyclonal Control DNA)
+ 1	no template control (water)
+ 1	to correct for pipetting errors
n = 2 × # of samples + 4	**Total**

- Therefore, the total aliquot volume for each master mix is **n × 45 µL**.
- For the Specimen Control Size Ladder master mix, the number of reactions (**m**) is:

m = # of samples	(run each sample in singlicate)
+ 1	negative control DNA (IVS-0000 Polyclonal Control DNA)
+ 1	no template control (water)
+ 1	to correct for pipetting errors
m = # of samples + 3	**Total**

- Therefore the total aliquot volume for the Specimen Control Size Ladder master mix is **m × 45 µL**.

7.3.3. **For *TCRB* Tubes A and B:** Add 2.25 U (or 0.45 µL @5 U/µL) of Taq DNA polymerase per reaction to each master mix.

- The total Taq DNA polymerase added to each master mix is **n × 0.45 µL**.
- Gently vortex to mix.

7.3.4. **For *TCRB* Tube C and *TCRG* Tubes A and B and Specimen Control Size Ladder:** Add 1.25 U (or 0.25 µL at 5 U/µL) of Taq DNA polymerase per reaction.

- The total Taq DNA polymerase added to each master mix is **n × 0.25 µL** for the *TCRB* Tube C master mix and **m × 0.25 µL** for the Specimen Control Size Ladder master mix.
- Gently vortex to mix.

7.3.5. For each reaction, aliquot 45 µL of the appropriate master mix + DNA polymerase solution into individual wells in a PCR plate or tube.

7.3.6. Add 5 µL of appropriate template (sample DNA, positive control DNA, negative control DNA or water) to the individual wells containing the respective master mix solutions. Pipette up and down several times to mix.

7.3.7. Cap or cover the PCR plate.

- Samples are now ready to be amplified on a thermal cycler.
- If amplification cannot be performed immediately following reagent preparation, the PCR plate or tubes can be stored at 2°C to 8°C for up to 24 hours.

Quick Guide

For each master mix and **n** reactions, mix:

n × 45 µL	Master Mix
n × 0.25 µL or 0.45 µL*	Taq DNA polymerase

Vortex gently to mix.

Aliquot **45 µL** of master mix + DNA polymerase solution into each reaction well.

Add **5 µL** of appropriate Template to each well.

Total reaction volume = **50 µL**

***Note:** Use **0.45 µL** of Taq DNA polymerase for *TCRB* Tubes A and B and **0.25 µL** of Taq DNA polymerase for *TCRB* Tube C and Specimen Control Size Ladder

7.4. Amplification

7.4.1. Amplify the samples using the following PCR program:

- Use the **calculated** option for temperature measurement with the BioRad PTC thermal cyclers.

Table 4. Thermal cycling conditions

Step	Temperature	Duration	Cycles
1	95°C	7 minutes	1
2	95°C	45 seconds	35
3	60°C	45 seconds	
4	72°C	90 seconds	
5	72°C	10 minutes	1
6	15°C	∞	1

7.4.2. Remove the amplification plate or tubes from the thermal cycler.

- Although amplified DNA is stable at room temperature for extended periods of time, store PCR products at 2°C to 8°C until detection. Detection must be within 30 days of amplification.

7.5. Gel Detection – Heteroduplex Analysis

- Do not heteroduplex the PCR products from the Specimen Control Size Ladder master mix. Skip steps 7.5.1-7.5.3 and proceed with step 7.5.4.

7.5.1. Denature 20 µL of PCR products at 94ºC for 5 minutes.

7.5.2. Quick chill to re-anneal PCR products at 4ºC (on an ice water bath) for 60 minutes.

7.5.3. Assemble the electrophoresis unit using a non-denaturing, 6% polyacrylamide TBE gel and 1X TBE running buffer.

7.5.4. Mix 20 µL of each sample with 5 µL of ice-cold non-denaturing 5X bromophenol blue loading buffer.

7.5.5. Load all 20 µL of the mixture into individual wells of the gel.

7.5.6. Run gel at 110V for 2-3 hours or 40-50V overnight. Voltage and electrophoresis time depend on the PCR amplicon size and polyacrylamide gel thickness. Voltage and run time can be adapted accordingly.

7.5.7. Stain gels in 0.5 µg/mL ethidium bromide (in water or 0.5X TBE Buffer) for 5-10 minutes.

7.5.8. De-stain gels in water for 5-10 minutes. Repeat with fresh water.

7.5.9. Place gel over UV illuminator to visualize bands.

7.5.10. Photograph and interpret resulting data. (See sections 8 *Sample Interpretation* and 10 *Expected Values*)

7.6. Quality Control

Positive and negative (or normal) controls are furnished with the kit and run in singlicate each time the assay is performed to ensure proper performance of the assay. In addition a no template control (*e.g.,* water) is also included to test for contamination of the master mix or cross-contamination of PCR reactions due to improper sterile technique. A buffer control may also be added to ensure that no contamination of the buffer used to resuspend the samples has occurred. The values for the positive controls are provided under section *10.1 Expected Size of Amplified Products*. Additional controls and sensitivity controls (dilutions of positive controls into our negative control) are available from Invivoscribe.

7.7. Recommended Positive Controls

The amplicon sizes listed were determined using an ABI platform.

Table 5. Recommended Positive Controls

Master Mix	Target	Control DNA	Catalog #	Product Size (bp)
***TCRB* Tube A**	Vβ + Jβ1/2	**Valid Size Range** IVS-0009 Clonal Control DNA	--- 40880490	**240 - 285** 264
***TCRB* Tube B**	Vβ + Jβ2	**Valid Size Range** IVS-0004 Clonal Control DNA IVS-0021 Clonal Control DNA	--- 40880190 40881210	**240 - 285** 253 267
***TCRB* Tube C**	Dβ + Jβ1/2	**Valid Size Range** IVS-0009 Clonal Control DNA	--- 40880490	**170 - 210 (Dß2), 285 - 325 (Dß1)** 309
***TCRG* Tube A**	Vγ1-8, Vγ10 + multiple Jγ regions	**Valid Size Range** IVS-0021 Clonal Control DNA	--- 40881210	**145 - 255** 211 (Vγ1-8 + Jγ 1.3/2.3)
***TCRG* Tube B**	Vγ9 Vγ11 + multiple Jγ regions	**Valid Size Range** IVS-0021 Clonal Control DNA	--- 40881210	**80 - 220** 167 (Vγ9 + Jγ 1.3/2.3)
Specimen Control Size Ladder	Multiple Genes	**Valid Size Range** IVS-0000 Polyclonal Control DNA	--- 40920010	**100, 200, 300, 400, 600**[a] 100, 200, 300, 400, 600[a]

[a]**Note:** Because smaller PCR fragments are preferentially amplified, it is not unusual for the 600 bp fragment to have a diminished signal or to be missing entirely.

8. Interpretation of Results

Although positive results are highly suggestive of malignancy, interpret both positive and negative results in the context of all clinical information and laboratory test results. The size range for each of the master mixes has been determined by testing positive and negative control samples. For accurate and meaningful interpretation it is important to ignore peaks that occur outside of the valid size range for each of the master mixes.

8.1. Analysis

- Report samples that fail to amplify following repeat testing as "**A result cannot be reported on this specimen because there was DNA of insufficient quantity or quality for analysis**".
- Repeat samples that test negative if the positive control reaction failed.
- If samples run in duplicate yield differing results, re-test and/or re-evaluat the samples for sample switching.
- **All assay controls must be examined prior to interpretation of sample results.**

Table 6. The following describes the analysis of each of the controls and the decisions necessary based upon the results.

Type of Control	Expected Result	Aberrant Result
No Template Control	No amplification present, continue with analysis	Amplification present. Repeat the assay.
Polyclonal Control	Product size is consistent with expected size listed in section *10.1 Expected Size of Amplified Products*. No clonal rearrangements are present. Continue with analysis.	Clonal rearrangements are present. Repeat the assay
Positive Control (This can also be an extraction control if positive control material is taken through extraction processes)	Product size is consistent with expected size listed in section *10.1 Expected Size of Amplified Products*. Continue with analysis.	Product size is not within the valid size range. Repeat the assay.
Specimen Control Size Ladder (This amplification control is essential for samples of unknown quantity and quality.)	If all of the 100, 200, 300, 400, and 600 bp peaks are seen, continue with analysis. Because smaller PCR fragments are preferentially amplified, it is not unusual for the 600 bp fragment to have a diminished signal or to be missing entirely. Continue with analysis.	If no bands are seen, repeat the assay unless specimen tests positive. If only 1, 2, or 3 bands are seen, re-evaluate sample for DNA degradation unless specimen tests positive.

8.2. Sample Interpretation

Given that the controls produce expected results, interpret the clinical samples as follows:

- One or two prominent positive bands[a] within the valid size range are reported as:

  "**Positive for the detection of clonal T cell receptor beta chain or gamma chain gene rearrangement(s), consistent with the presence of a clonal cell population."** In the context of overall diagnostic criteria, clonal cell populations can indicate the presence of hematologic malignancy.

- An absence of positive bands[a] within the valid size range is reported as:
  "**Negative for the detection of clonal T cell receptor beta chain or gamma chain gene rearrangement(s)**."

[a]**Note:** **Criteria for defining a positive band are as follows:**

- Products generated from samples that fall within the valid size range and produce a discrete band(s) distinct from any background smear are consistent with a positive band.

9. Limitations of Procedure

- This assay does not identify 100% of clonal cell populations.
- This assay cannot reliably detect less than five (5) positive cells per 100 normal cells.
- Always interpret the results of molecular clonality tests in the context of clinical, histological and immunophenotypic data.
- PCR-based assays are subject to interference by degradation of DNA or to inhibition of PCR due to EDTA, heparin and other agents.

10. Expected Values

10.1. Expected Size of Amplified Products

The amplicon sizes listed were determined using an ABI platform.

Table 7. Expected Size of Amplified Products

Master Mix	Target	Control DNA	Catalog #	Product Size in (bp)
***TCRB* Tube A**	Vβ + Jβ1/2	**Valid Size Range** IVS-0000 Polyclonal Control DNA IVS-0009 Clonal Control DNA IVS-0004 Clonal Control DNA	--- 40920010 40880490 40880190	**240 - 285** 240 - 285, 271[a] 264 295
***TCRB* Tube B**	Vβ + Jβ2	**Valid Size Range** IVS-0000 Polyclonal Control DNA IVS-0009 Clonal Control DNA IVS-0004 Clonal Control DNA IVS-0021 Clonal Control DNA	--- 40920010 40880490 40880190 40881210	**240 - 285** 240 - 285, 221[b] --- 253 267
***TCRB* Tube C**	Dβ + Jβ1/2	**Valid Size Range** IVS-0000 Polyclonal Control DNA IVS-0009 Clonal Control DNA IVS-0004 Clonal Control DNA	--- 40920010 40880490 40880190	**170 - 210 (Dβ2), 285 - 325 (Dβ1)** 128[b], 170 - 210, 285 - 325, 337[b] 309 295
***TCRG* Tube A**	Vγ1-8, Vγ10 + multiple Jγ regions	**Valid Size Range** IVS-0000 Polyclonal Control DNA IVS-0009 Clonal Control DNA IVS-0021 Clonal Control DNA	--- 40920010 40880490 40881210	**145 - 255** 230 - 255 (Vγ1-8 + Jγ 1.1/2.1), 195 - 230 (Vγ1-8 + Jγ 1.3/2.3), 175 - 195 (Vγ10 + Jγ 1.1/2.1), 145 - 175 (Vγ10 + Jγ 1.3/2.3) 212 (Vγ1-8 + Jγ 1.3/2.3) 211 (Vγ1-8 + Jγ 1.3/2.3)
***TCRG* Tube B**	Vγ9 Vγ11 + multiple Jγ regions	**Valid Size Range** IVS-0000 Polyclonal Control DNA IVS-0009 Clonal Control DNA IVS-0021 Clonal Control DNA	--- 40920010 40880490 40881210	**80 - 220** 195 - 220 (Vγ9 + Jγ 1.1/2.1), 160 - 195[c] (Vγ9 + Jγ 1.3/2.3), 110 - 140[d] (Vγ11 + Jγ 1.1/2.1), 80 - 110[d] (Vγ11 + Jγ 1.3/2.3) 115[e] (Vγ11 + Jγ 1.3/2.3) 167 (Vγ9 + Jγ 1.3/2.3)
Specimen Control Size Ladder	Multiple Genes	**Valid Size Range** IVS-0000 Polyclonal Control DNA	--- 40920010	**100, 200, 300, 400, 600[f]** 100, 200, 300, 400, 600[f]

[a]**Note:** The 271 bp band is particularly seen in samples with low numbers of contaminating lymphoid cells.

[b]**Note:** Under sub-optimal conditions nonspecific products of 221 bp (in Tube B) and 128 and 337 bp (in Tube C) can be detected. If present, these bands will normally be faint.

[c]**Note:** Amplicon product is often not seen in this size range.

[d]**Note:** Amplicon product is often not seen in this size range. This is an extremely restricted repertoire.

[e]**Note:** This may be seen as a weak amplicon.

[f]**Note:** Because smaller PCR fragments are preferentially amplified, it is not unusual for the 600 bp fragment to have a diminished signal or to be missing entirely.

10.2. Sample Data

The data shown in Figures 2 - 7 were generated using the master mixes indicated. Amplified products were run on a 6% polyacrylamide gel.

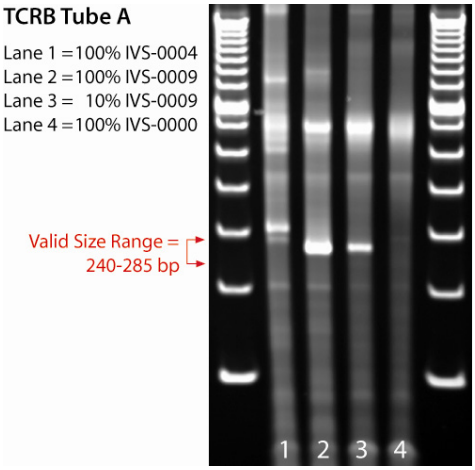

Figure 2. *TCRB* Tube A

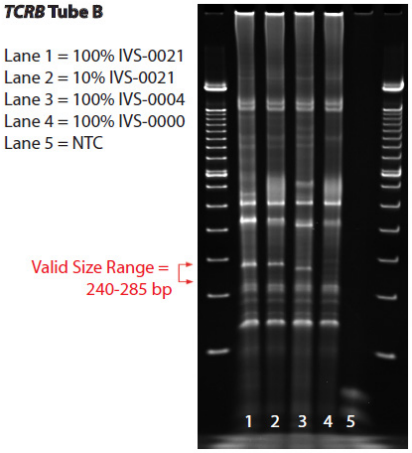

Figure 3. *TCRB* Tube B

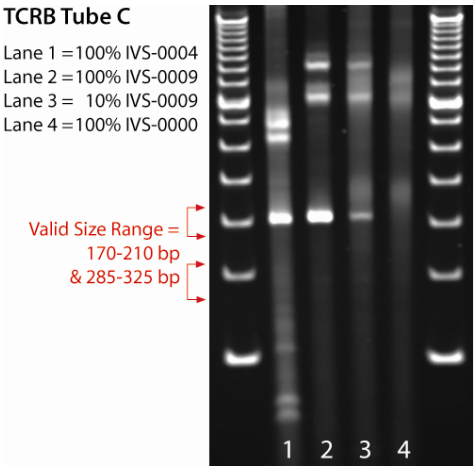

Figure 4. *TCRB* Tube C

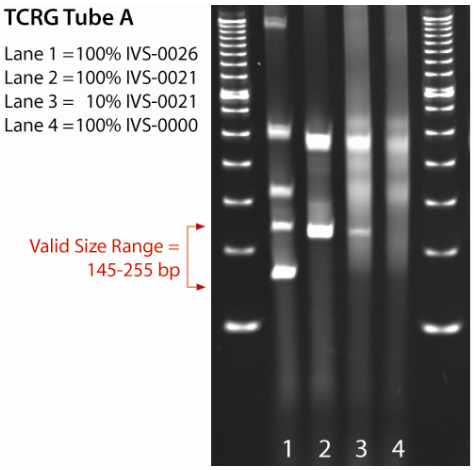

Figure 5. *TCRG* Tube A

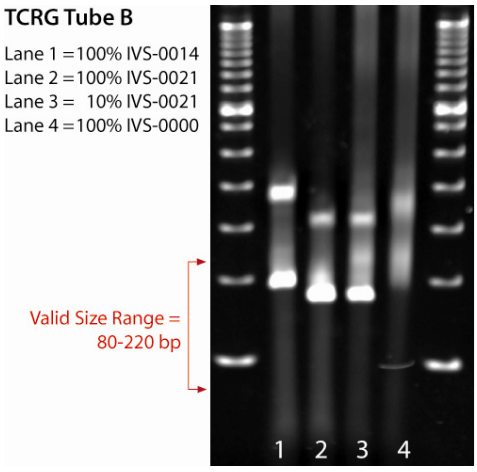

Figure 6. *TCRG* Tube B – 6FAM & HEX

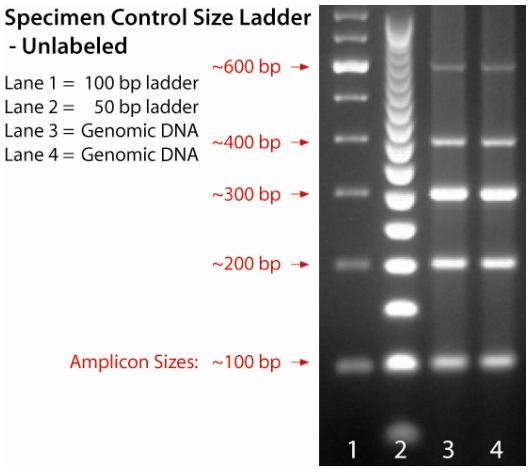

Figure 7. Products were amplified using the Specimen Control Size Ladder master mix then run on a 2% agarose gel.

11. Performance Characteristics

This IdentiClone *TCRB* + *TCRG* T-Cell Clonality Assay is a rapid and reliable procedure that is far more sensitive than Southern Blot (SB) analysis in detecting clonality in suspect lymphoproliferations. The final clinical histopathological diagnosis correlates well with PCR results in a higher number of patients in comparison with SB results. This is evidenced by two notable papers, one published in 2003 in *Leukemia* by van Dongen *et al.* and one published in 2005 in the *Journal of Molecular Diagnostics* (*JMD*) by Sandberg *et al*.

Table 8. Comparison of PCR and Southern Blot detection

PCR/SB concordance (*Leukemia*):[2]		PCR/SB concordance (*JMD*):[3]	
IGH:	93% sensitivity/ 92% specificity	*IGH* + *IGK*:	85% sensitivity
IGK:	90% sensitivity/ 90% specificity		
IGL:	86% sensitivity/ 92% specificity		
TCRB:	86% sensitivity/ 98% specificity	*TCRB*:	85% sensitivity
TCRG:	89% sensitivity/ 94% specificity		
TCRD:	83% sensitivity/ 95% specificity		

Table 9. PCR vs. SB analysis relative to histopathology and final diagnosis

	PCR/SB concordance:	PCR sensitivity:	SB sensitivity
IGH + *IGK*:	85%	98%	39%
TCRB:	85%	96%	35%

The study by Sandberg *et al.* was an independent study of 300 patient samples from a variety of sample types. In cases where both PCR and SB analyses were done and results could be correlated with histopathology and a final diagnosis, the diagnostic accuracy of selected IdentiClone tests was determined to be at least 96%. This was far more accurate than SB analysis, which in this study missed 23 clear cases of malignancy and 7 probable malignancies. There were no clear false positive results generated using the IdentiClone tests and there was a high level of precision.[3] In addition a clear benefit of this assay was that clonal results generated allowed for subsequent detection of patient- and tumor-specific gene rearrangements for minimal residual disease detection.

12. Technical and Customer Service

Technical and Customer Service Representatives are available Monday through Friday to answer phone, e-mail or website inquiries.

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1. Miller, JE, Wilson, SS, Jaye, DJ, Kronenberg, M. (1999). An automated semiquantitative B and T cell clonality assay. *Molecular Diagnostics* 4, 101-117.
2. Van Dongen, JJM *et al*. (2003). Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: Report of the BIOMED-2 Concerted Action BMH4-CT98-3936. *Leukemia*. 17, 2257-2317.
3. Sandberg, Y, *et al*. (2005). BIOMED-2 multiplex immunoglobulin/T-cell receptor polymerase chain reaction protocols can reliably replace Southern Blot analysis in routine clonality diagnostics. *J. Moecular Diagnostic* 7, 495-503.
4. van Krieken, JHJM *et al*. (2007). Improved reliability of lymphoma diagnostics via PCR-based clonality testing: – Report of the BIOMED-2 Concerted Action BHM4-CT98-3936. *Leukemia* 21, 201-206.

14. Symbols

The following symbols are now used in labeling for Invivoscribe diagnostic products.

IVD	For *In Vitro* Diagnostic Use		Expiration Date
REF	Catalog Number	EC REP	Authorized Representative in the European Community
VOL	Reagent Volume		Storage Conditions
LOT	Lot Number		Consult Instructions for Use

15. Legal Notice

15.1. Warranty and Liability

Invivoscribe, Inc. (Invivoscribe®) is committed to providing the highest quality products. Invivoscribe® warrants that the products meet or exceed the performance standards described in the Instructions For Use, as to products with such an insert. If a product is covered by product specifications and does not perform as specified, our policy is to replace the product or credit the full purchase price. No other warranties of any kind, expressed or implied, are provided by Invivoscribe®. Invivoscribe® liability shall not exceed the purchase price of the product. Invivoscribe shall have no liability for direct, indirect, consequential or incidental damages arising from the use, results of use, or inability to use its products; product efficacy under purchaser controlled conditions in purchaser's laboratory must be established and continually monitored through purchaser defined and controlled processes including but not limited to testing of positive, negative, and blank controls every time a sample is tested. Ordering, acceptance, and use of product constitutes purchaser acceptance of sole responsibility for assuring product efficacy and purchaser agreement to the limitation of liability set forth in this paragraph.

This product is an in vitro diagnostic product is not available for sale or use within North America.

15.2. Patents and Trademarks

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Identiclone® is a registered trademark of Invivoscribe®

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