

Instructions for Use

CE IVD

**IdentiClone® TCRG Gene Clonality Assay**For Identification of Clonal T Cell Receptor Gamma (*TCRG*) chain gene rearrangements**IVD** For *In Vitro* Diagnostic Use🔑 Storage Conditions: **-85°C to -65°C**

(DNA controls may be separated from assay kits and stored at 2°C to 8°C)

Catalog#	Products	Quantity
<b>REF</b> 92070020	IdentiClone <i>TCRG</i> Gene Clonality Assay – Gel Detection	33 Reactions
<b>REF</b> 92070040	IdentiClone <i>TCRG</i> Gene Clonality Assay MegaKit – Gel Detection	330 Reactions

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## 1. Intended Use

The IdentiClone *TCRG* Gene Clonality Assay 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 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

## 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.<sup>1</sup> This PCR assay employs multiple consensus DNA primers that target conserved genetic regions within the T-cell receptor gamma chain gene. This assay is used to detect the vast majority of clonal T-cell malignancies from DNA, and test products can be analyzed using a variety of detection formats, including gel and capillary electrophoresis.

Invivoscribe's 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 performed at more than thirty prominent independent testing centers throughout Europe in a collaborative study known as the BIOMED-2 Concerted Action.<sup>2</sup>

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.**

### 2.2. Summary

This test kit includes three (3) master mixes. The *TCRG* Tube A master mix contains primers that target the V $\gamma$ 1-8 + V $\gamma$ 10 genes and J $\gamma$ 1.1, J $\gamma$ 1.3, J $\gamma$ 2.1, and J $\gamma$ 2.3 genes (also known as J $\gamma$ P1, J $\gamma$ 1, J $\gamma$ P2, and J $\gamma$ 2, respectively). The *TCRG* Tube B master mix contains primers that target the V $\gamma$ 9 + V $\gamma$ 11 genes and J $\gamma$ 1.1, J $\gamma$ 1.3, J $\gamma$ 2.1, and J $\gamma$ 2.3 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 which improves consistency and facilitates cross training on a broad range of different assays.

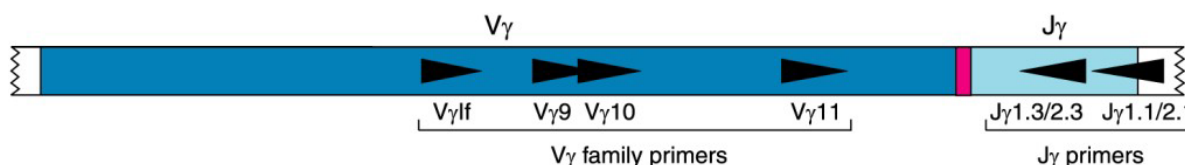
This assay is based on the EuroClonality/BIOMED-2 Concerted Action BMH4-CT98-3936.



### 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 conserved regions within the variable (V) and joining (J) regions that flank the unique hypervariable antigen-binding region 3 (CDR3). 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 observed. DNA from samples containing a clonal population yield one or two prominent amplified products (amplicons) within a diminished polyclonal background.



**TCRG Tube A: V $\gamma$ 1f and V $\gamma$ 10 primers + J $\gamma$ 1.1/2.1 and J $\gamma$ 1.3/2.3**

**TCRG Tube B: V $\gamma$ 9 and V $\gamma$ 11 primers + J $\gamma$ 1.1/2.1 and J $\gamma$ 1.3/2.3**

**Figure 1.** This is a simplified diagram of a representative rearranged 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 Tubes A and B. (The V $\gamma$ 1f primer is a consensus primer that targets V $\gamma$ 1 through V $\gamma$ 8).

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 V 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).


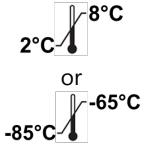
## 4. Reagents

### 4.1. Reagent Components

**Table 1:** Available Assays

Catalog #	Description	Quantity
<b>REF</b> 92070020	IdentiClone <i>TCRG</i> Gene Clonality Assay – Gel Detection	33 Reactions
<b>REF</b> 92070040	IdentiClone <i>TCRG</i> Gene Clonality Assay MegaKit – Gel Detection	330 Reactions

**Table 2:** Kit Components

Reagent	Catalog # ( <b>REF</b> )	Reagent Components (active ingredients)	Unit Quantity	92070020 # of Units	92070040 # of Units	Storage Temp.
<b>Master Mixes</b>	22070030CE	<b><i>TCRG</i> Tube A – Unlabeled</b> Multiple oligonucleotides targeting the V $\gamma$ 1-8, V $\gamma$ 10, and multiple J $\gamma$ regions of the T-cell receptor gamma gene in a buffered salt solution.	1500 $\mu$ L	1	10	
	22070040CE	<b><i>TCRG</i> Tube B – Unlabeled</b> Multiple oligonucleotides targeting the V $\gamma$ 9, V $\gamma$ 11, and multiple J $\gamma$ regions of the T-cell receptor gamma gene in a buffered salt solution.	1500 $\mu$ L	1	10	
<b>Template Amplification Control Master Mix</b>	20960020	<b>Specimen Control Size Ladder – Unlabeled</b> Multiple oligonucleotides targeting housekeeping genes.	1500 $\mu$ L	1	10	
<b>Positive Control DNAs</b>	40880490	<b>IVS-0009 Clonal Control DNA</b> 200 $\mu$ g/mL of DNA in 1/10 <sup>th</sup> TE solution	100 $\mu$ L	1	5	
	40881210	<b>IVS-0021 Clonal Control DNA</b> 200 $\mu$ g/mL of DNA in 1/10 <sup>th</sup> TE solution	100 $\mu$ L	1	5	
<b>Negative (Normal) Control DNA</b>	40920010	<b>IVS-0000 Polyclonal Control DNA</b> 200 $\mu$ g/mL of DNA in 1/10 <sup>th</sup> TE solution	100 $\mu$ L	1	5	

**Note:** There are no preservatives used in the manufacture of this kit.

## 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. Use care to ensure use of correct thermal cycler program, 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.
- 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. Follow uni-directional workflow 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

- For any duration other than immediate use, **store assay kits at -85°C to -65°C**.
- The optimum storage temperature for DNA controls is 2°C to 8°C, but DNA controls can be stored long term 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
- 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** (gDNA) 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)
- 2 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<sup>th</sup> 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 gDNA at 2°C to 8°C or at -85°C to -65°C for long term storage.



## 7. Assay Procedure

### 7.1. Materials Provided

**Table 3:** Kit components

Catalog #	Description
<b>REF</b> 22070030CE	<i>TCRG</i> Tube A – Unlabeled
<b>REF</b> 22070040CE	<i>TCRG</i> Tube B – Unlabeled
<b>REF</b> 20960020	Specimen Control Size Ladder – Unlabeled
<b>REF</b> 40880490	IVS-0009 Clonal Control DNA
<b>REF</b> 40881210	IVS-0021 Clonal Control DNA
<b>REF</b> 40920010	IVS-0000 Polyclonal Control DNA

### 7.2. Materials Required (not provided)

**Table 4:** Materials Required (not provided)

Reagent/Material	Recommended Reagents/Materials and Suppliers	Catalog #	Notes
<b>DNA Polymerase</b>	Roche: <ul style="list-style-type: none"> <li>EagleTaq DNA Polymerase</li> </ul> Invivoscribe, Inc.: <ul style="list-style-type: none"> <li>EagleTaq DNA Polymerase<sup>1</sup> or equivalent</li> </ul>	05206944190 60970100	N/A
<b>Glass Distilled De-ionized Molecular Biology Grade or USP Water</b>	N/A	N/A	Sterile and free of DNase and RNase.
<b>Calibrated Pipettes</b>	Rainin: <ul style="list-style-type: none"> <li>P-2, P-20, P-200, and P-1000 pipettes</li> <li>Or SL-2, SL-20, SL-200, and SL-1000 pipettes</li> </ul>	N/A	Must be able to accurately measure volumes between 1 µL and 1000 µL.
<b>Thermal cycler</b>	Bio-Rad: <ul style="list-style-type: none"> <li>MJ Research PTC-100 or PTC-200, PTC-220, PTC-240</li> </ul> Perkin-Elmer <ul style="list-style-type: none"> <li>PE 9600 or PE 9700</li> </ul>	N/A	N/A
<b>Vortex Mixer</b>	N/A	N/A	N/A
<b>PCR plates or tubes</b>	N/A	N/A	Sterile
<b>Filter barrier pipette tips</b>	N/A	N/A	Sterile, RNase/DNase/Pyrogen-free
<b>Microcentrifuge tubes</b>	N/A	N/A	Sterile
<b>Gel Electrophoresis Unit</b>	N/A	N/A	For polyacrylamide gels
<b>Ethidium Bromide</b>	Thermo Fisher Scientific: <ul style="list-style-type: none"> <li>UltraPure™ 10 mg/mL Ethidium Bromide</li> </ul>	15585-011	N/A
<b>6% Polyacrylamide Gels</b>	Thermo Fisher Scientific: <ul style="list-style-type: none"> <li>Novex® TBE Gels (6%, 12 well)</li> </ul>	EC62652Box	N/A
<b>TBE Running Buffer</b>	Thermo Fisher Scientific: <ul style="list-style-type: none"> <li>Novex TBE Running Buffer (5X)</li> </ul>	LC6675	Dilute 1:5 prior to use.
<b>Gel Loading Buffer</b>	Thermo Fisher Scientific: <ul style="list-style-type: none"> <li>10X BlueJuice™ Gel Loading Buffer</li> <li>Novex Hi-Density TBE Sample Buffer (5X)</li> </ul>	10816-015 LC6678	N/A
<b>100 bp DNA Ladder</b>	Thermo Fisher Scientific: <ul style="list-style-type: none"> <li>TrackIt™ 100 bp DNA Ladder</li> </ul>	10488-058	N/A

<sup>1</sup>**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 to ensure 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, **duplicate** testing is recommended 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 with each master mix.

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  $\mu\text{L}$  for each reaction.
- Include 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:

<b>n = 2 × # of samples</b>	(run each sample in duplicate)
+ 1	positive control DNA (See section 7.7 <i>Recommended Positive Controls</i> )
+ 1	negative control DNA (IVS-0000 Polyclonal Control DNA)
+ 1	no template control (water)
+ 1	to correct for pipetting errors
<hr/>	
<b>n = 2 × # of samples + 4</b>	<b>Total</b>

- Therefore the total aliquot volume for each master mix is **n × 45  $\mu\text{L}$** .
- For the Specimen Control Size Ladder master mix, the number of reactions (**m**) is:

<b>m = # of samples</b>	(run each sample in duplicate)
+ 1	positive control DNA (IVS-0000 Polyclonal Control DNA)
+ 1	no template control (water)
+ 1	to correct for pipetting errors
<hr/>	
<b>m = # of samples + 3</b>	<b>Total</b>

- Therefore the total aliquot volume for the Specimen Control Size Ladder master mix is **m × 45  $\mu\text{L}$** .

7.3.3. Add 1.25 units (or 0.25  $\mu\text{L}$  at 5 U/ $\mu\text{L}$ ) of Taq DNA polymerase per reaction to each master mix.

- The total Taq DNA polymerase added to each master mix is **n × 0.25  $\mu\text{L}$** , and **m × 0.25  $\mu\text{L}$**  for the Specimen Control Size Ladder master mix.
- Gently vortex to mix.

7.3.4. For each reaction, aliquot 45  $\mu\text{L}$  of the appropriate master mix + DNA polymerase solution into individual wells in a PCR plate or tube.

7.3.5. Add 5  $\mu\text{L}$  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.

7.3.6. 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  $\mu\text{L}$  Master Mix

**n** × 0.25  $\mu\text{L}$  Taq DNA polymerase

Vortex gently to mix.

Aliquot **45  $\mu\text{L}$**  of master mix + DNA polymerase solution into each reaction well.

Add **5  $\mu\text{L}$**  of appropriate Template to each well

Total reaction volume = **50  $\mu\text{L}$**

## 7.4. Amplification

7.4.1. Amplify the samples using the following PCR program:

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

**Table 5:** 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 – 7.5.2 and proceed with step 7.5.3.

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

7.5.2. Quick chill to re-anneal PCR products at 4°C (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: *Interpretation of Results* and 10: *Expected Values*)

## 7.6. Quality Control

Positive and negative (or normal) controls are furnished with the kit and can be run in singlicate each time the assay is performed to ensure proper performance of the assay. In addition, include a no template control (*e.g.*, water) to test for contamination of the master mix or cross-contamination of reactions. 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 in 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 6:** Recommended Positive Controls

Master Mix	Target	Control DNA	Catalog #	Product Size in base pairs (bp)
<b>TCRG Tube A</b>	V $\gamma$ 1-8, V $\gamma$ 10 + multiple J $\gamma$ regions	<b>Valid Size Range</b> IVS-0021 Clonal Control DNA	--- 40881210	<b>145 - 255</b> 211 (V $\gamma$ 1-8 + J $\gamma$ 1.3/2.3)
<b>TCRG Tube B</b>	V $\gamma$ 9, V $\gamma$ 11 + multiple J $\gamma$ regions	<b>Valid Size Range</b> IVS-0021 Clonal Control DNA	--- 40881210	<b>80 - 220</b> 167 (V $\gamma$ 9 + J $\gamma$ 1.3/2.3)
<b>Specimen Control Size Ladder</b>	Multiple Genes	<b>Valid Size Range</b> IVS-0000 Polyclonal Control DNA	--- 40920010	<b>100, 200, 300, 400, 600<sup>a</sup></b> 100, 200, 300, 400, 600 <sup>a</sup>

<sup>a</sup>**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, both positive and negative results in the context of all clinical information and laboratory test results. The size range for each master mix 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 master mix.

### 8.1. Analysis

- 8.1.1. 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**”.
- 8.1.2. Repeat testing on samples that test negative if the positive control reaction failed.
- 8.1.3. If samples run in duplicate yield differing results, re-test and/or re-evaluate samples for sample switching.
- 8.1.4. All assay controls must be examined prior to interpretation of sample results. If the controls do not yield the correct results, the assay is not valid and the samples cannot be interpreted.

**Table 7:** 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
<b>No Template Control</b>	No amplification present, continue with analysis	Amplification present, repeat the assay.
<b>Polyclonal Control</b>	Product size is consistent with expected size listed in section 10.1: <i>Expected Size of Amplified Products</i> . No clonal rearrangements are present. Continue with analysis.	Clonal rearrangements are present. Repeat the assay
<b>Positive Control</b> (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: <i>Expected Size of Amplified Products</i> . Continue with analysis.	Repeat the assay.
<b>Specimen Control Size Ladder</b> (This amplification control is <u>essential</u> 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 <u>unless specimen tests positive</u> . If only 1, 2, or 3 bands are seen, re-evaluate sample for DNA degradation <u>unless specimen tests positive</u> .

## 8.2. Sample Interpretation

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

- 8.2.1. One or two prominent positive bands<sup>a</sup> within the valid size range are reported as:
- **“Positive for the detection of clonal T cell receptor 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.”**
- 8.2.2. An absence of positive bands<sup>a</sup> within the valid size range is reported as:
- **“Negative for the detection of clonal T cell receptor gamma chain gene rearrangement(s).”**

<sup>a</sup>**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 8:** Recommended Positive Controls

Master Mix	Target	Control DNA	Catalog #	Product Size in base pairs (bp)
<b>TCRG Tube A</b>	V $\gamma$ 1-8, V $\gamma$ 10 + multiple J $\gamma$ regions	<b>Valid Size Range</b>	---	<b>145 - 255</b>
		IVS-0000 Polyclonal Control DNA	40920010	30-255 (V $\gamma$ 1-8 + J $\gamma$ 1.1/2.1)
				195-230 (V $\gamma$ 1-8 + J $\gamma$ 1.3/2.3)
				175-195 (V $\gamma$ 10 + J $\gamma$ 1.1/2.1)
				145-175 (V $\gamma$ 10 + J $\gamma$ 1.3/2.3)
<b>TCRG Tube B</b>	V $\gamma$ 9, V $\gamma$ 11 + multiple J $\gamma$ regions	<b>Valid Size Range</b>	---	<b>80 - 220</b>
		IVS-0000 Polyclonal Control DNA	40920010	195-220 (V $\gamma$ 9 + J $\gamma$ 1.1/2.1),
				160-195 <sup>a</sup> (V $\gamma$ 9 + J $\gamma$ 1.3/2.3),
				110-140 <sup>b</sup> (V $\gamma$ 11 + J $\gamma$ 1.1/2.1),
				80-110 <sup>b</sup> (V $\gamma$ 11 + J $\gamma$ 1.3/2.3)
<b>Specimen Control Size Ladder</b>	Multiple Genes	<b>Valid Size Range</b>	---	<b>100, 200, 300, 400, 600<sup>a</sup></b>
		IVS-0000 Polyclonal Control DNA	40920010	100, 200, 300, 400, 600 <sup>a</sup>

<sup>a</sup>**Note** : Amplicon product is often not seen in this size range.

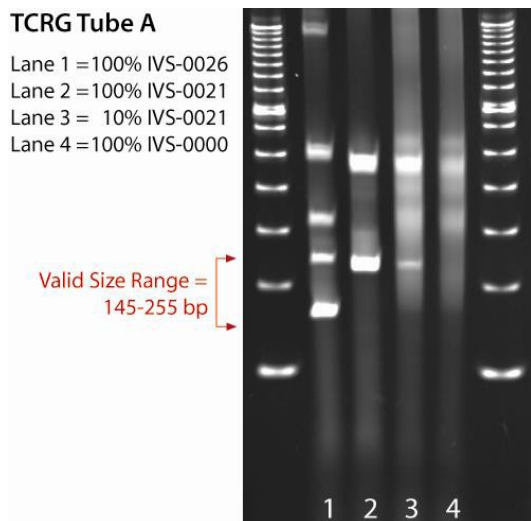
<sup>b</sup>**Note** : Amplicon product is often not seen in this size range. This is an extremely restricted repertoire.

<sup>c</sup>**Note** : This may be seen as a weak amplicon.

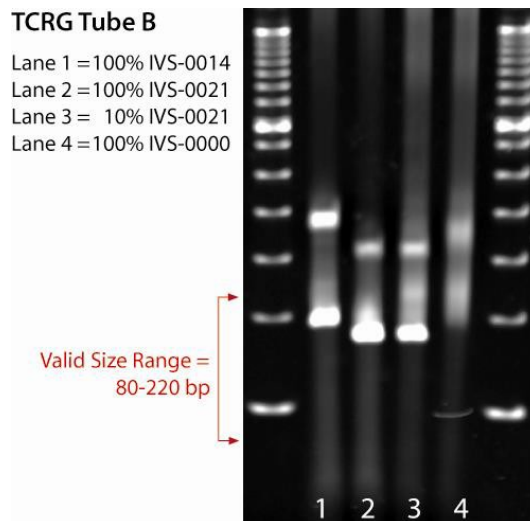
<sup>d</sup>**Note** : Because smaller PCR fragments are preferentially amplified, it is not unusual for the 600 bp fragment to have a diminished signal or to be missing entirely.

## 10.2. Sample Data

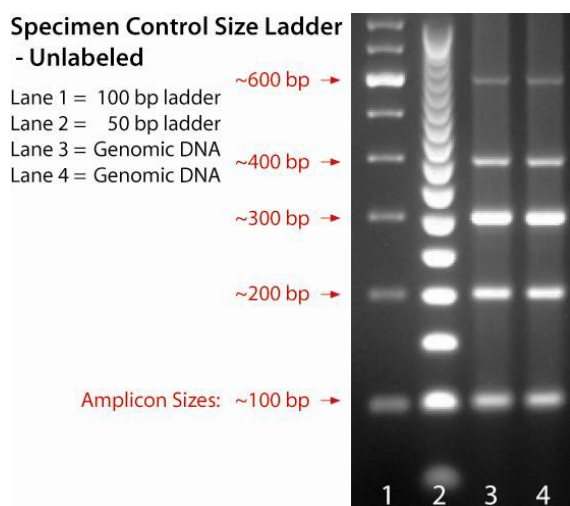
The data shown below were generated using the master mixes indicated. Amplified products were heteroduplexed and run on a 6% polyacrylamide gel.



**Figure 2.** TCRG Tube A master mix.



**Figure 3.** TCRG Tube B master mix.



**Figure 4.** Specimen Control Size Ladder master mix.

## 11. Performance Characteristics

This IdentiClone TCRG Gene Clonality PCR 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 clinico-histopathological diagnosis correlates well with PCR results in a higher number of patients in comparison with SB results.<sup>2</sup>

**Table 9.** Concordance Studies

PCR/SB concordance: <sup>2</sup>	
<i>IGH</i> :	93% sensitivity/ 92% specificity
<i>IGK</i> :	90% sensitivity/ 90% specificity
<i>IGL</i> :	86% sensitivity/ 92% specificity
<i>TCRB</i> :	86% sensitivity/ 98% specificity
<i>TCRG</i> :	89% sensitivity/ 94% specificity
<i>TCRD</i> :	83% sensitivity/ 95% specificity

The diagnostic accuracy of this test was determined to be at least 89%. There were no clear false positive results generated using the IdentiClone tests and there was a high level of precision. 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. Bibliography

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2. Van Dongen, JJM *et al.* 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* 2003, **17(12)**:2257-2317.
3. van Krieken, JHJM, *et al.* Improved reliability of lymphoma diagnostics via PCR-based clonality testing: report of the BIOMED-2 Concerted Action BHM4-CT98-3936. *Leukemia* 2007, **21(2)**:201-206.

## 13. Technical and Customer Service


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## 14. Symbols

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

	For <i>In Vitro</i> Diagnostic Use		Expiration Date
	Catalog Number		Authorized Representative in the European Community
	Reagent Volume		Manufacturer
	Lot Number		Consult Instructions for Use
	Storage Conditions		



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