Instructions for Use

RUO

T-Cell Receptor Gamma Gene Rearrangement Assay

For Identification of T-Cell Clonality.

RUO This assay is for Research Use Only. Not for use in diagnostic procedures.

Manufactured in U.S.A.

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 12070051	T-Cell Receptor Gamma Gene Rearrangement Assay - ABI Fluorescence Detection	30 Reactions

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1. Assay Use

This Research Use Only assay identifies T-Cell Receptor Gamma gene rearrangements and is useful for the study of clonal T-cell populations and evaluation of new research and methods in malignancy studies.

2. Summary and Explanation of the Test

2.1. Background

Rearrangement of the variable (Vγ) and joining (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]

The T-cell receptor gamma (*TRG*, formerly known as *TCRG*) chain locus spans 160 kilobases on chromosome 7 (7p14). The locus consists of 14 Vγ gene segments in six (6) subgroups, and five (5) Jγ gene segments interspersed between two (2) constant (Cγ) gene segments. However, the repertoire of functional *TRG* molecules is limited to 4-6 functional Vγ gene segments that belong to two (2) subgroups.[2]

2.2. Summary

Invivoscribe's gene rearrangement and translocation assays represent a simple approach to PCR-based testing and utilize a series of standardized master mixes that have been carefully optimized. Several master mixes are used to test for rearrangements at each receptor locus and each master mix targets a different conserved region within the receptor gene. This comprehensive testing approach maximizes identification of clonal rearrangements. A single universal thermal cycler program and similar detection methodologies are used with all of our tests; this improves consistency and facilitates cross-training.

This test kit includes three (3) master mixes. *TCRG* Mix 1 and *TCRG* Mix 2 target framework regions within the variable (V) region and the joining (J) region of the *TCR* gamma chain locus. The Amplification Control master mix targets the *HLA-DQα* locus and generates an amplicon of 235 base pairs (bp) to ensure the quality and quantity of input DNA is adequate to yield a valid result.

3. Principles of the Procedure

3.1. Polymerase Chain Reaction (PCR)

Polymerase chain reaction (PCR) assays are routinely used for the identification of clonal B- and T-cell populations. These tests amplify the DNA between primers that target the conserved framework (FR) and joining (J) regions. 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 and light chains genes 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 this region is amplified using DNA primers that flank this region, a clonal population of cells yields one or two prominent amplified products (amplicons) within the expected size range. Two products are generated in cases when the initial rearrangement was non-productive and was followed by rearrangement of the other homologous chromosome. In contrast, DNA from a normal or polyclonal (many clones) population produces a bell-shaped curve of amplicon products (or Gaussian distribution) that reflect the heterogeneous population of V-J region rearrangements.

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 smear of different-sized amplicon products that form a Gaussian distribution around a statistically favored, average-sized rearrangement. Please see Figure 1 for a diagram representing the organization of a rearranged T-cell receptor gamma chain gene.

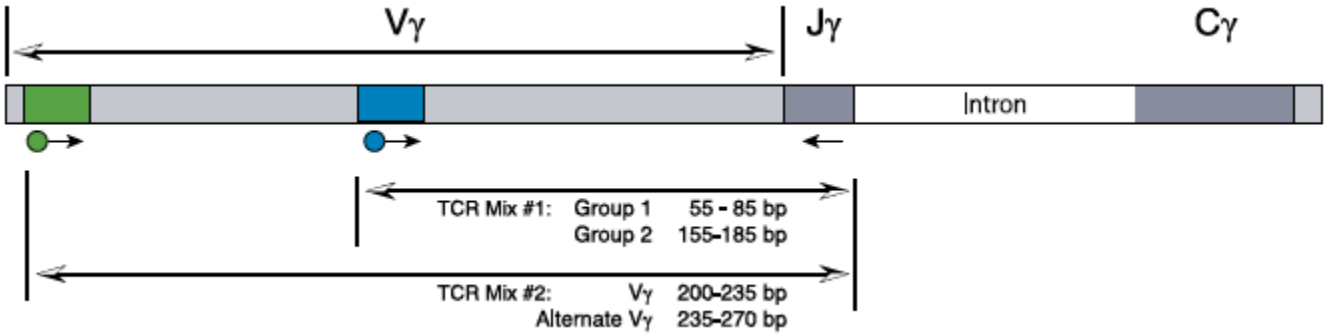

Figure 1. This is a diagrammatic representation of the T-cell receptor gamma chain gene on chromosome 7. The location of the variable (V), joining (J) and constant (C) regions are indicated. Colored arrows represent conserved regions within the variable region gene segments targeted by primers. Primers 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.

3.2. Differential Fluorescence Detection

Differential fluorescence detection is commonly used to resolve the different-sized amplicon products using a capillary electrophoresis instrument. Primers can be conjugated with several different fluorescent dyes (fluorophores) so that they can 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 unsurpassed sensitivity, single nucleotide resolution, differential product detection and relative quantification. In addition, the use of agarose and polyacrylamide gels, as well as the use of carcinogens such as ethidium bromide, can virtually be eliminated. Further, differential detection allows accurate, reproducible and objective interpretation of primer-specific products and automatic archiving of data. Inter-assay and intra-assay reproducibility in size determination using capillary electrophoresis is approximately 1 to 2 base pair (bp). This reproducibility and sensitivity coupled with the automatic archiving of specimen data allows for the monitoring, tracking and comparison of data over time.

4. Reagents

4.1. Reagent Components

Table 1. Available Kits

Catalog #	Description	Total Reactions
REF 12070051	T-Cell Receptor Gamma Gene Rearrangement Assay - ABI Fluorescence Detection	30 Reactions

Table 2. Reagent Components

Reagent	Catalog # (REF)	Reagent Components (active ingredients)	Unit of Quantity	# of Units	Storage Temp.
Control and Standard	40880490	**IVS-0009 Clonal Control DNA** 200 µg/mL of DNA in 1/10th TE solution	100 µL	1	2°C – 8°C or -85°C – -65°C
	40920010	**IVS-0000 Polyclonal Control DNA** 200 µg/mL of DNA in 1/10th TE solution	100 µL	1	
Differential Fluorescence Detection					
Master Mixes	22070071	**T-Cell Receptor Gamma Mix 1 – 6FAM** Multiple oligonucleotides targeting the Vγ1-8 + Vγ9 + Jγ1/2 consensus regions of the T-cell receptor gamma gene in a buffered salt solution.	1500 µL	1	-85°C – -65°C
	22070021	**T-Cell Receptor Gamma Mix 2 - HEX** Multiple oligonucleotides targeting the alternate Vγ + Jγ1/2 consensus regions of the T-cell receptor gamma gene in a buffered salt solution.	1500 µL	1	
Template Amplification Control Master Mix	20960011	**Amplification Control Master Mix – 6FAM** Multiple oligonucleotides targeting the *HLA-DQα* locus.	1500 µL	1	

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

4.2. Warnings and Precautions

- Use this assay kit as a system; do not substitute other manufacturers' reagents. Dilution, reducing amplification reactions or other deviations from this protocol may affect the performance of this test and/or nullify any limited sublicense that come with the purchase of these kits.
- Do not mix or combine reagents from kits with different lot numbers.
- Materials are stable until the labeled expiration date when stored and handle as directed. Do not use kits beyond their expiration date.
- Adherence to the protocol will assure optimal performance and reproducibility. Ensure correct thermal cycler programs are used, as other programs may provide inaccurate/faulty data such as false-positive and false-negative results.
- Dispose of unused reagents and waste in accordance with country, federal, state and local regulations.
- Perform all laboratory procedures with standard personal protective equipment (gloves, laboratory coats and protective eyewear). Follow good laboratory practices and universal precautions when working with specimens. Do not pipette by mouth. Do not eat, drink or smoke in laboratory work areas. Wash hands thoroughly after handling specimens and assay reagents. Handle specimens in approved biological safety containment facilities and open only in certified biological safety cabinets.
- Due to the high analytical sensitivity of this test, use extreme care to avoid any contamination of reagents or amplification mixtures with samples, controls or amplified materials. Use fresh, aerosol-resistant pipette tips between samples and between dispensing reagents. Closely monitor all reagents for signs of contamination (*e.g.,* negative controls giving positive signals). Discard any reagents suspected of contamination.
- To minimize contamination, wear clean gloves when handling samples and reagents and routinely clean work areas and pipettes prior to setting up PCR.
- Follow uni-directional workflow between separate work areas in the PCR laboratory: begin with master mix preparation, move to specimen preparation, then to amplification and finally to detection. Autoclaving does not eliminate DNA contamination. Perform pre- and post-PCR steps in separate spaces. Avoid taking paper and other materials from post-PCR into the pre-PCR space.
- Dedicate all pipettes, pipette tips and any equipment used in a particular area to that area of the laboratory.
- Decontaminate non-disposable items with 10% bleach and rinse with distilled water two separate times before returning them to the starting areas.
- Use sterile, disposable plastic ware whenever possible to avoid contamination.

4.3. Storage and Handling

- Store the assay at **-85ºC to -65ºC** until ready to use.
- All reagents and controls must be thawed and vortexed or mixed thoroughly prior to use to ensure that they are completely resuspended.
- Due to high salt concentrations, PCR master mixes are sensitive to freeze/thaw cycles. Minimize the exposure of master mixes to freeze/thaw cycles.

For any questions, please contact the Invivoscribe technical staff. We would be happy to help determine optimal storage needs.

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. ABI Capillary Electrophoresis Instruments

- Use or function: Fragment detection and analysis
- Performance characteristics and specification:
  - The following capillary electrophoresis instruments will meet the performance needs for this assay:
    - ABI 310 Genetic Analyzer (1-capillary)
    - ABI 3100 Avant Genetic Analyzer (4-capillaries)
    - ABI 3100 Genetic Analyzer (16-capillaries)
    - ABI 3130 Genetic Analyzer (4-capillaries)
    - ABI 3130xL Genetic Analyzer (16-capillaries)
    - ABI 3500 Genetic Analyzer (8-capillaries)
    - ABI 3500xL Genetic Analyzer (24-capillaries)
- Follow manufacturer's installation, operation, calibration, and maintenance procedures.
- The ABI instrument used must be calibrated with appropriate Matrix Standards as outlined in section 7.2. *Materials Required (not provided).*
- Use the default settings for each polymer and capillary type.
- See section 6.4. *Sample Preparation* for sample preparation.

6. Specimen Collection and Preparation

6.1. Precautions

Biological specimens from humans may contain potentially infectious materials. Handle all specimens in accordance with your institute's Bloodborne Pathogen program and/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 extracted and purified genomic DNA (gDNA). Common sources of gDNA include:

- 5 cc of peripheral blood, bone marrow biopsy or bone marrow aspirate anti-coagulated with heparin or EDTA. Ship at ambient temperature; OR
- Formalin-fixed paraffin embedded tissue or slides

6.4. Sample Preparation

- Test all samples in **singlicate**.
- Include **positive, negative and no template controls** with each master mix tested.

6.4.1. Using any method of DNA extraction, extract the gDNA from unknown samples.

6.4.2. Resuspend DNA to final concentration of 100 µg – 400 µg per mL in TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) or distilled 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.
- When testing unknown samples, a template amplification control master mix (*e.g.,* Amplification Control or Specimen Control Size ladder) can be used to verify the absence of PCR inhibitors and sufficient quality and quantity of DNA is present to generate a valid result.

6.5. Template Amplification Control

The **Amplification Control** master mix primers are available unlabeled for gel detection and labeled with a fluorescent dye (6-FAM) for ABI fluorescence detection. The 6-FAM label is detected as **BLUE** using the differential fluorescence software. The amplicons produced with this master mix are 235 bp.

- Run the products of this master mix separately.

6.6. Sample Storage

Store samples using a method that prevents degradation of DNA.

7. Assay Procedure

7.1. Materials Provided

Table 3. Materials Provided

Catalog #	Description
REF 22070071	T-Cell Receptor Gamma Mix 1 – 6FAM
REF 22070021	T-Cell Receptor Gamma Mix 2 – HEX
REF 20960011	Amplification Control – 6FAM
REF 40880490	IVS-0009 Clonal Control DNA
REF 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	Part Number	Notes
DNA Polymerase	Roche: • EagleTaq DNA Polymerase Invivoscribe, Inc. • FalconTaq DNA Polymerase	05206944190 60970130	N/A
Molecular Biology Grade or USP Water	N/A	N/A	DNase / RNase free
Calibrated Pipettes	N/A	N/A	Capable of accurately measuring volumes between 0.5 µL and 1000 µL.
Thermal cycler	Thermo Fisher Scientific: • Veriti Dx Thermal Cycler Bio-Rad: • MJ Research PTC-100 or PTC-200, PTC-220, PTC-240 Perkin-Elmer • PE 2600, 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
ABI Fluorescence Detection			
ABI Capillary Electrophoresis Instrument	Thermo Fisher Scientific: • ABI 310, 3100, or 3500 series	N/A	N/A
Hi-Di Formamide	Thermo Fisher Scientific: • Hi-Di™ Formamide	4311320	N/A
Size Standards	Invivoscribe, Inc.: ○ Hi-Di Formamide w/ROX size standards for ABI 310 ○ Hi-Di Formamide w/ROX size standards for ABI 3100 Thermo Fisher Scientific: • For ABI 3100 or 3130 instruments: ○ GeneScan™ - 400HD [ROX]™ • For ABI 3500 instruments: ○ GeneScan - 600 [LIZ]™ v2.0	60980051 60980061 402985 4408399	N/A

Table 4. Materials Required (not provided)

Reagent/Material	Recommended Reagents/Materials and Suppliers	Part Number	Notes
Spectral Calibration Dye Sets	Thermo Fisher Scientific: • For ABI 3100 and 3130 instruments: ○ DS-30 Matrix Standard Kit (Dye Set D) • For ABI 310 instruments: ○ NED Matrix Standard ○ And Fluorescent Amidite Matrix Standards [6FAM, TET, HEX, TAMRA, ROX] • For ABI 3500 instruments: ○ DS-33 Matrix Standard Kit (Dye Set G5)	 4345827 402996 401546 4345833	N/A
Polymer	Thermo Fisher Scientific: • POP-4™ Polymer: ○ POP-4 for 310 Genetic Analyzers ○ POP-4 for 3100/3100-Avant Genetic Analyzers ○ POP-4 for 3130/3130xL Genetic Analyzers • POP-7™ Polymer: ○ POP-7 for 3130/3130xL Genetic Analyzers ○ POP-7 for 3500/3500xL Genetic Analyzers	 402838 4316355 4352755 4352759 4393714	N/A
Buffer	Thermo Fisher Scientific: ○ 10X Genetic Analyzer Buffer with EDTA	 402824	Dilute 1:10 in sterile water before use

7.3. Reagent Preparation

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

7.3.2. In containment hood or dead air box, aliquot the appropriate volume of each master mix into a clean, sterile microfuge tube.

- Aliquot volumes are 50 µL for each sample + 150 µL (3 x 50 µL) for the positive, negative and no template controls.
- Add an additional 20 µL to correct for pipetting errors.

7.3.3. Add the appropriate amount of Taq DNA polymerase (0.25 µL @5 U/ µL per 55 µL total reaction volume) to each master mix and gently mix by inverting several times or gently vortexing.

- The master mixes are now ready for distribution to reaction tubes or plate and addition of sample.

7.4. Amplification

7.4.1. Aliquot 50 µL of the master mix/enzyme solutions into individual PCR wells or tubes.

7.4.2. Add 5 µL of sample or control DNA to the individual tubes or wells containing the respective master mix reactions. Pipette up and down several times to mix.

7.4.3. Amplify the reactions using the appropriate PCR program in Table 5.

- Use the **calculated** option for temperature measurement with the PTC instruments.

Table 5. Thermal cycling conditions

Step	Temperature	Duration	Cycles
1	95°C	**7 minutes**	1
2	94°C	30 seconds	35
3	55°C	30 seconds	
4	72°C	1 minute	
5	72°C	10 minutes	1
6	4°C	∞	1

7.4.4. Remove the amplification plate from the thermal cycler.

7.5. Detection

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ABI Fluorescence Detection with ABI 310, 3100 & 3130 instruments

7.5.1. In a new microcentrifuge tube, mix an appropriate amount (10 µL per reaction) of Hi-Di Formamide with ROX Size Standards.[a] Vortex well.

7.5.2. In a new 96-well PCR plate, add 10 µL of Hi-Di Formamide with ROX size standards to individual wells for each reaction.

7.5.3. Transfer 1 µL of each reaction to the wells containing Hi-Di Formamide with ROX size standards.

- Add only one sample per well.
- Pipette up and down to mix.

7.5.4. Cap or cover the PCR plate.

7.5.5. Heat denature the samples at 95ºC for 2 minutes, then snap chill on ice for 5 minutes.

7.5.6. Prepare a **sample sheet** and **injection list** for the samples.

7.5.7. Run the samples on an ABI 310/3100/3130 capillary electrophoresis instrument according to its user manual.

7.5.8. Review profile and controls, report results.

- Data are automatically displayed as size and color specific peaks.

ABI Fluorescence Detection with ABI 3500 instruments

Note: Due to instrument to instrument variation in the performance of the ABI 3500 platform, the amount of formamide, sample and size standard listed in the protocol is intended to be a starting point. The protocol may need to be optimized for specific ABI 3500 Platforms.

7.5.9. In a new microcentrifuge tube, mix an appropriate amount (9.5 µL per reaction) of Hi-Di Formamide with LIZ Size Standards.[a] Vortex well.

7.5.10. In a new 96-well PCR plate, add 9.5 µL of Hi-Di Formamide with LIZ size standards to individual wells for each reaction.

7.5.11. Transfer 0.5 µL of each reaction to the wells containing Hi-Di Formamide with LIZ size standards.

- Add only one sample per well.
- Pipette up and down to mix.

7.5.12. Cap or cover the PCR plate.

7.5.13. Heat denature the samples at 95ºC for 3 minutes, then snap chill on ice for 5 minutes.

7.5.14. Prepare a **sample sheet** and **injection list** for the samples.

7.5.15. Run the samples on an ABI 3500 capillary electrophoresis instrument according to its user manual.

7.5.16. Review profile and controls, report results.

- Data are automatically displayed as size and color specific peaks.

[a]Note: Please see the accompanying Applied Biosystems product insert for mixing Hi-Di Formamide with size standards for different ABI instruments.

7.6. Recommended Positive Controls

Table 6. Recommended positive controls and size of amplified products

Master Mix	Target	Color	Control DNA	Catalog #	Product Size (bp)
T-Cell Receptor Gamma Mix 1	Vγ1-8, 9 + J1/2 consensus	**Blue**	**Valid Size Range – Group I** **Valid Size Range – Group II** IVS-0009 Clonal Control DNA	--- --- 40880490	**55 – 85** **155 – 185** 60[1], 68
T-Cell Receptor Gamma Mix 2	Alternate Vγ + J1/2	**Green**	**Valid Size Range – Group I** **Valid Size Range – Group II** IVS-0009 Clonal Control DNA	--- --- 40880490	**200 – 235[2]** **235 – 270[2]** 216[1], 248
Amplification Control	HLA-DQα	**Blue**	**Valid Size Range** IVS-0000 Polyclonal Control DNA	--- 40920010	**235** 235

Note: The amplicon sizes listed above were determined using an ABI platform. Amplicon sizes observed on each specific CE instrument may differ 1-4 bp from those listed above depending on the platform of detection (ABI) and the version of the analysis software used. Once identified, the amplicon size as determined on the specific platform used will be consistent from run to run.

[1]Note: These are generally observed as peakswith weak amplitudes.

[2]Note: This multiplex master mix generates two distinct size ranges of products (200-235 bp and 235-270 bp). A clonal sample will frequently generate a clonal product in each of these size ranges. For example, the positive control generates peaks of 216 and 248 bp. When products are generated in both of these size ranges the difference in size of these peaks is approximately 32 bp.

8. Interpretation and Reporting

This assay is for research use only; not intended for diagnostic purposes. PCR based testing does not identify 100% of clonal cell populations; therefore, repeat testing by NGS may be advisable to rule out clonality.

The size range for each master mix has been determined by testing positive control samples and gDNA. For accurate and meaningful interpretation ignore peaks that occur outside of the valid size range for each master mix. Peaks that are outside of the range cannot be assumed to be valid.

8.1. Sample Analysis

Results can be reported as "**Positive**" or "**Negative**" for "**Detection of clonal T-cell receptor gamma chain gene rearrangement, consistent with the presence of a clonal cell population**."

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. All assay controls must be examined prior to interpretation of sample results.

The following describes the analysis of each control and the decisions necessary based upon the results.

- **Negative Control**: (Polyclonal control, water or no template blank). If the negative control is:
  - **Positive**: Possible contamination of all PCR amplification reactions. Do not continue with the interpretation of results. Prepare fresh master mix and repeat amplification.
  - **Negative**: Continue with the analysis.
- **Positive Control**: (This can also be an extraction control if positive control material is taken through extraction processes). If the positive control is:
  - **Positive:** Continue with analysis.
  - **Negative:** Repeat the assay.
- **Amplification Control**: (This can be run on unknown samples). If the Amplification Control :
  - **Generates 235 bp product**: Continue with analysis.
  - **Does not generate specified product**: Repeat the assay and/or re-extract the specimen.

8.2. Sample Interpretation

Following the acceptance of the controls, the samples are interpreted as follows: One or two prominent bands within the valid size range are reported as **"Detection of clonal T-cell receptor gamma chain gene rearrangement, consistent with the presence of a clonal cell population."**

9. Limitations of Procedure

The assay is subject to interference by degradation of DNA or inhibition of PCR due to heparin or other agents.

10. Expected Results

10.1. Expected Size of Amplified Products

Note: "**Color**" indicates the color of products generated with the master mix when using differential fluorescence detection format (*e.g.*, ABI instruments).

Table 7. Expected Size of Amplified Products

Master Mix	Target	Color	Control DNA	Cat#	Product Size (bp)
T-Cell Receptor Gamma Mix 1	Vγ1-8,9 + Jγ1/2	**Blue**	**Valid Size Range – Group I** **Valid Size Range – Group II** IVS-0000 Polyclonal Control DNA IVS-0009 Clonal Control DNA	--- --- 40920010 40880490	**55 - 85** **155 - 185** 55 - 85, 155 - 185 60[Δ], 68
T-Cell Receptor Gamma Mix 2	Alt. Vγ+ Jγ1/2	**Green**	**Valid Size Range – Group I** **Valid Size Range – Group II** IVS-0000 Polyclonal Control DNA IVS-0009 Clonal Control DNA	--- --- 40920010 40880490	**200 - 235[ᵾ]** **235 - 270[ᵾ]** 200 - 270 216[Δ], 248
Amplification Control	HLA-DQα	**Blue**	**Valid Size** IVS-0000 Polyclonal Control DNA	--- 40920010	**235** 235

Note: The amplicon sizes listed above were determined using an ABI platform. Amplicon sizes observed on each specific CE instrument may differ 1-4 bp from those listed above depending on the platform of detection (ABI, etc.) and the version of the analysis software used. Once identified, the amplicon size as determined on the specific platform used will be consistent from run to run.

[Δ]Note: These are generally observed as peakswith weak amplitudes.

[ᵾ]Note: This multiplex master mix generates two distinct size ranges of products (200-235 bp and 235-270 bp). A clonal sample will frequently generate a clonal product in each of these size ranges. For example, the positive control generates peaks of 216 and 248 bp. When products are generated in both of these size ranges the difference in size of these peaks is approximately 32 bp.

10.2. Sample Data

ABI Fluorescence Detection: The data shown in Figures 2 -4 were generated using the master mixes indicated. Amplified products were run on an ABI instrument.

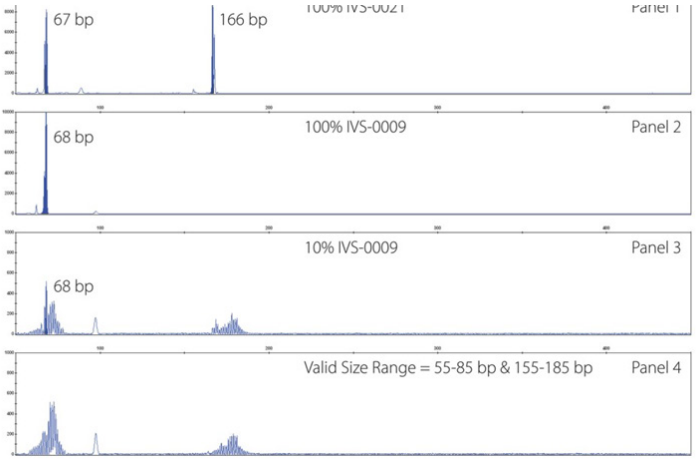

Figure 2. Amplified products using the T-Cell Receptor Gamma Mix 1 – 6FAM. (left)

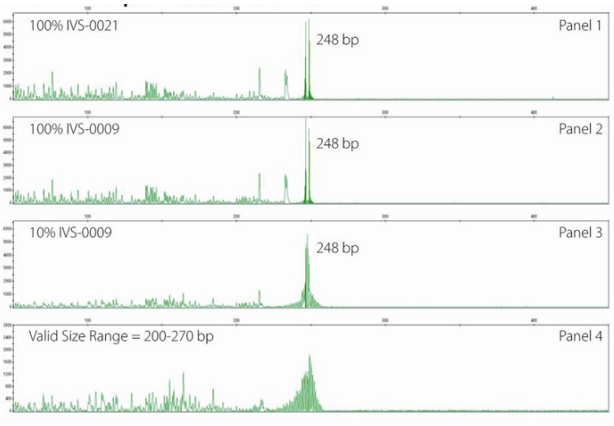

Figure 3. Amplified products using the T-Cell Receptor Gamma Mix 2 – HEX. (right)

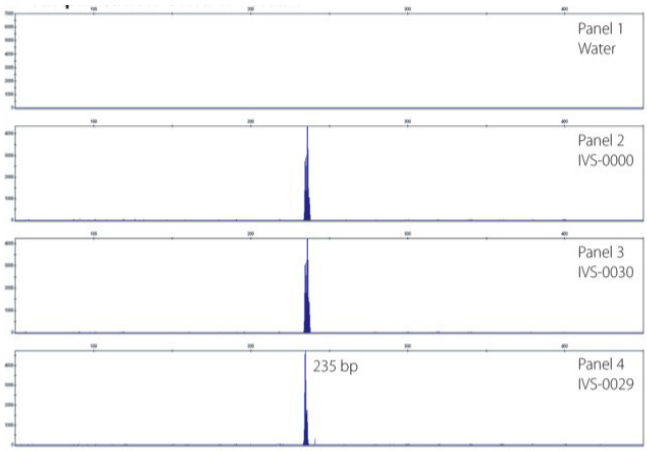

Figure 4. Data was generated by the Amplification Control master mix. (left)

11. Technical and Customer Service

Thank you for purchasing our ***TCRG* Gene Rearrangement Assay**. We appreciate your business. We are happy to assist you in the validation of this assay and will provide ongoing technical assistance to keep the assays performing efficiently in your laboratory. Technical assistance is most rapidly obtained using our Internet site: **http://www.invivoscribe.com** or by sending an email inquiry to: **support@invivoscribe.com**.

Contact Information

Invivoscribe, Inc

10222 Barnes Canyon Road | Building 1 | San Diego | California 92121-2711 | USA

Phone: +1 858 224-6600 | Fax: +1 858 224-6601 | Business Hours: 7:00AM - 5:00 PM PST/PDT

Technical Service: support@invivoscribe.com | Customer Service: sales@invivoscribe.com | Website: www.invivoscribe.com

12. References

1. Miller, JE, Wilson, SS, Jaye, DJ, Kronenberg, M. An automated semiquantitative B- and T-cell clonality assay. *Mol. Diag*. 1999, **4(2)**:101-117.
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 JH, Langerak AW, Macintyre EA, Kneba M, Hodges E, Sanz RG, Morgan GJ, Parreira A, Molina TJ, Cabeçadas J, Gaulard P, Jasani B, Garcia JF, Ott M, Hannsmann ML, Berger F, Hummel M, Davi F, Brüggemann M, Lavender FL, Schuuring E, Evans PA, White H, Salles G, Groenen PJ, Gameiro P, Pott Ch, van Dongen JJM. Improved reliability of lymphoma diagnostics via PCR-based clonality testing: report of the BIOMED-2 Concerted Action BHM4-CT98-3936. *Leukemia*. 2007; **21(2)**:201-6.

13. Symbols

The following symbols are used in Invivoscribe product labeling.

REF	Catalog Number		Expiration Date
VOL	Reagent Volume		Manufacturer
LOT	Lot Number		Consult Instructions for Use
	Storage Conditions	RUO	Research Use Only

14. Legal Notice

14.1. Warranty and Liability

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This product is for Research Use Only, not for use in diagnostic procedures.

14.2. Patents and Trademarks

This product is covered by one or more of the following: European Patent Number 1549764, European Patent Number 2418287, European Patent Number 2460889, Japanese Patent Number 4708029, United States Patent 8859748, and related pending and future applications. All of these patents and applications are licensed exclusively to Invivoscribe®. Additional patents licensed to Invivoscribe covering some of these products apply elsewhere. Many of 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 this product.

15. T-Cell Receptor Gamma Gene Rearrangement Assay: Single Page Guide

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

15.2. In a containment hood or dead air box remove an appropriate aliquot to clean, sterile microfuge tube (one tube for each master mix). Aliquot volumes are 50 µL for each sample + 150 µL for the positive, negative and no template controls. Add an additional 20 µL to correct for pipetting errors.

15.3. Add the appropriate amount of Taq DNA polymerase (0.25 µL @5 U/ µL per 55 µL total reaction volume) to each master mix and gently mix by inverting several times or gently vortexing.

15.4. Aliquot 50 µL of master mix to individual wells of a PCR plate.

15.5. Add 5 µL of DNA from the unknown and control samples to individual tubes or wells containing the respective master mix reactions and pipette up and down several times to mix. Amplify target DNA using the universal thermal cycler program.

ABI Fluorescence Detection with ABI 310, 3100 & 3130 instruments

15.6. In a new microcentrifuge tube, mix an appropriate amount (10 µL per reaction) of Hi-Di Formamide with ROX Size Standards. Vortex well.

15.7. In a new 96-well PCR plate, add 10 µL of Hi-Di Formamide with ROX size standards to individual wells for each reaction.

15.8. Transfer 1 µL of each reaction to the wells containing Hi-Di Formamide with ROX size standards. Add only one sample per well. Pipette up and down to mix.

15.9. Cap or cover the PCR plate.

15.10. Heat denature the samples at 95ºC for 2 minutes, then snap chill on ice for 5 minutes.

15.11. Prepare a **sample sheet** and **injection list** for the samples.

15.12. Run the samples on an ABI 310/3100/3130 capillary electrophoresis instrument according to its user manual.

15.13. Review profile and controls, then report results.

ABI Fluorescence Detection with ABI 3500 instruments

15.14. In a new microcentrifuge tube, mix an appropriate amount (9.5 µL per reaction) of Hi-Di Formamide with LIZ Size Standards. Vortex well.

15.15. In a new 96-well PCR plate, add 9.5 µL of Hi-Di Formamide with LIZ size standards to individual wells for each reaction.

15.16. Transfer 0.5 µL of each reaction to the wells containing Hi-Di Formamide with LIZ size standards. Add only one sample per well. Pipette up and down to mix.

15.17. Cap or cover the PCR plate.

15.18. Heat denature the samples at 95ºC for 3 minutes, then snap chill on ice for 5 minutes.

15.19. Prepare a **sample sheet** and **injection list** for the samples.

15.20. Run the samples on an ABI 3500 capillary electrophoresis instrument according to its user manual.

15.21. Review profile and controls, then report results.