Instructions for Use

RUO

T-Cell Receptor Gamma Gene Rearrangement Assay 2.0

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	12070101	T-Cell Receptor Gamma Gene Rearrangement Assay 2.0	33 Reactions
REF	12070111	T-Cell Receptor Gamma Gene Rearrangement Assay 2.0 MegaKit	330 Reactions

Table of Contents

1. Assay Use

This Research Use Only assay identifies T-cell receptor gamma (*TCRG*) chain gene rearrangements and is useful for the identification of clonal T-cell populations and evaluation of new research and methods in malignancy studies.

2. Summary and Explanation of the Test

2.1. Background

Invivoscribe's T-Cell Receptor Gamma Gene Rearrangement Assay 2.0 represents an improved approach to PCR-based clonality testing of T-cell lymphoproliferative disorders. This assay was optimized using a single amplification reaction with a single fluorescent dye for detection. Amplified products generated targeting the *TCR* gamma gene locus all fall within a single size range to facilitate interpretation.

The human *TCR* gamma gene locus on chromosome seven (7q14) includes 14 V genes (six of these V genes are functional; three (3) open reading frames and five (5) pseudogenes) belonging to four subgroups (Group I, II, III and IV), five (5) J segments and two (2) C genes spread over 200 kilobases. The diversity of this locus has complicated PCR-based testing. This multiplex PCR assay represents an improvement over existing assays as it can detect the vast majority of *TCR* gamma gene rearrangements with a single multiplex master mix. Importantly, this assay includes primers for all known groups of *TCR* gamma variable region genes and joining region genes involved in rearrangements in T-cell lymphomas. In addition, all labeled primers are conjugated with the 6FAM fluorophore.

2.2. Summary

Our single multiplex master mix targets all conserved regions within the variable (V) and the joining (J) region genes that are described in lymphoid malignancies. This is critical for more comprehensive analysis of samples, as some T-cell lymphoproliferative disorders involve V and J segments that would not be identified with a single Vγ (1-8) and Jγ1/2 primer set. Amplification with all primers in a single tube has several additional important advantages over existing methods. The polyclonal background that results from the combination of all primers in a single tube produces a more robust and easily interpreted signal with capillary electrophoresis, which aids in the interpretation of small peaks. The average size of the *TCRG* gene rearrangement PCR product is 190 bp, with a normal distribution of product sizes between 159 and 207 bp. This protocol leads to improved PCR product formation from paraffin-embedded samples compared to other protocols that yield products of 260 bp or longer. Positive and negative DNA controls, as well as a Specimen Control Size Ladder master mix are included. Clonality is indicated if a dominant amplicon is detected.

This test kit includes the *TCRG* - 6FAM Master Mix that targets framework regions within the variable region and the joining region of the *TCR* gamma chain locus. The second master mix, the Specimen Control Size Ladder, targets multiple genes and generates a series of amplicons of 100, 200, 300, 400, and 600 bp that can be used to ensure that the quality of input DNA is adequate to yield a valid result.

3. Principles of the Procedure

3.1. Polymerase Chain Reaction (PCR)

PCR assays are routinely used for the identification of clonal T-cell populations. This test amplifies the DNA between primers that target conserved regions within the variable (V) and the 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 Gaussian distribution (bell-shaped curve) of amplicon products is produced within an expected size range. This Gaussian distribution reflects the heterogeneous population of V-J rearrangements. In certain cases, where lymphocyte DNA is not present, no product is detected. DNA from samples containing a clonal population yield one or two prominent amplified products (amplicons) within the valid size range.

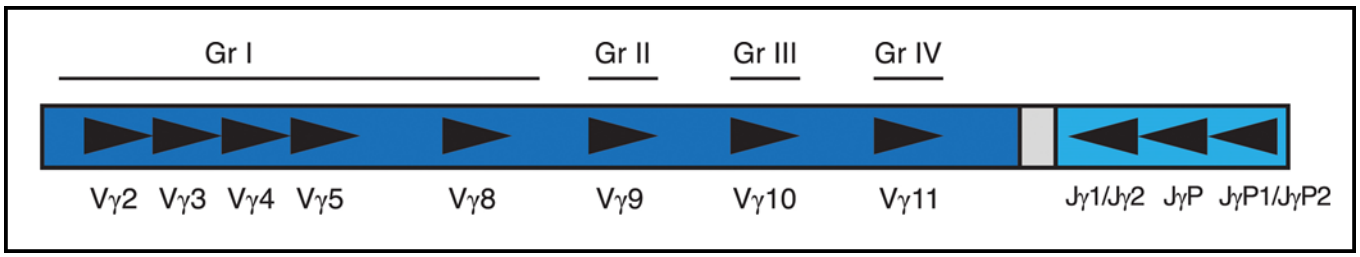

Figure 1: Simple representation of the organization if the T-cell receptor gamma gene on chromosome 7. Black arrows represent the relative positions of primers that target the variable region genes and the joining downstream joining region gene segments that are involved in rearrangements in T-cell lymphomas. The downstream primers are fluorescently labeled through the incorporation of a 6 FAM fluorophore. The amplicon products generated from these rearrangements are detected by capillary electrophoresis.

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, a multiplex master mix, which targets multiple V and J regions (Figure 1), is required to detect the majority of clonal rearrangements. As indicated, clonal rearrangements are identified as one or two prominent, single-sized products within the background of different-sized amplicon products that form the 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 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.

3.3. 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 one to two nucleotides.

4. Reagents

4.1. Reagent Components

Table 1. Available Kits

Catalog #		Description	Total Reactions
REF	12070101	T-Cell Receptor Gamma Gene Rearrangement Assay 2.0	33 Reactions
REF	12070111	T-Cell Receptor Gamma Gene Rearrangement Assay 2.0 MegaKit	330 Reactions

Table 2. Reagent Components

Reagent	Catalog #	Reagent Components (active ingredients)	Unit Quantity	12070101 # of Units	12070111 # of Units	Storage Temp.
Master Mixes	22070091	***TCRG* – 6FAM** Multiple oligonucleotides targeting the Vγ2, 3, 4, 5, 8, 9, 10, & 11 and Jγ1/Jγ2, JγP, and JγP1/JγP2 regions of the T-cell receptor gamma gene in a buffered salt solution.	1500 µL	1	10	-85°C -65°C
Template Amplification Control Master Mix	20960021	**Specimen Control Size Ladder – 6FAM** Multiple oligonucleotides targeting housekeeping genes.	1500 µL	1	10	
Positive Control DNA	40883320	**5% *TCRG* Positive Control DNA** 50 µg/mL of DNA in 1/10th TE solution	50 µL	1	5	2°C 8°C or -85°C -65°C
Negative (Normal) Control DNA	40920020	***TCRG* Negative Control DNA** 50 µg/mL of DNA in 1/10th TE solution	50 µL	1	5	

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 handled 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.

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

5. Instruments

5.1. Thermal cycler

- Use or function: Amplification of DNA samples
- Performance characteristics and specification:
  - Minimum thermal range: 15ºC to 96ºC
  - Minimum ramping speed: 0.8ºC/sec
- Follow manufacturer's installation, operation, calibration, and maintenance procedures.
- See section 7.4. *Amplification* for thermal cycler program.

5.2. Electrophoresis Unit (for Gel Detection)

- 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 (for Gel Detection)

- 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.

5.4. 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 Avant Genetic Analyzer (4-capillaries)
    - 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. *: Reagents Required (not provided).*
- Use the default settings for your polymer and capillary type.
- See section 7.5. *Detection*.

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

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; OR
- Formalin-fixed paraffin embedded tissue or slides.

6.4. Sample Preparation

- Test all samples in **duplicate**.
- **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 20 µg – 200 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, it is recommended to use a template amplification control master mix (*e.g.*, Specimen Control Size Ladder) 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 Controls

The **Specimen Control Size Ladder** master mix primers are labeled with a fluorescent dye (6-FAM). This label is detected as **BLUE** using the differential fluorescence software. The amplicons produced with this master mix are at ~100, 200, 300, 400, and 600 bp. Please note that the ~100 bp band is comprised of 84 bp and 96 bp bands. Both of these bands co-migrate on a gel. 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

See Table 2 for materials provided.

7.2. Reagents Required (not provided)

Table 3. Materials Required (not provided)

Reagent/Material	Recommended Reagents/Materials and Suppliers	Part Number	Notes
DNA Polymerase	Roche: • EagleTaq DNA Polymerase or equivalent	05206944190	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 1 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 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 Detection			
Ethidium Bromide	Thermo Fisher Scientific: • UltraPure™ 10 mg/mL Ethidium Bromide	15585-011	N/A
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
Gel Electrophoresis Unit	N/A	N/A	N/A
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	Part Number	Notes
	ABI Fluorescence Detection		
ABI Capillary Electrophoresis Instrument	Thermo Fisher Scientific: • ABI 310, 3100, 3130, or 3500 series	N/A	N/A
Hi-Di Formamide	Thermo Fisher Scientific: • Hi-Di™ Formamide	4311320	N/A
Size Standards	Invivoscribe: • 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: o GeneScan™ - 400HD [ROX]™ • For ABI 3500 instruments: o GeneScan - 600 [LIZ]™ v2.0	60980051 60980061 402985 4408399	N/A
Buffer	Thermo Fisher Scientific: • 10X Genetic Analyzer Buffer with EDTA	402824	Dilute 1:10 in sterile water before use
Spectral Calibration Dye Sets	Thermo Fisher Scientific: • For ABI 3100 and 3130 instruments: o DS-30 Matrix Standard Kit (Dye Set D) • For ABI 310 instruments: o NED Matrix Standard o And Fluorescent Amidite Matrix Standards [6FAM, TET, HEX, TAMRA, ROX] • For ABI 3500 instruments: o DS-33 Matrix Standard Kit (Dye Set G5)	4345827 402996 401546 4345833	N/A
Polymer	Thermo Fisher Scientific: • POP-4™ Polymer: o POP-4 for 310 Genetic Analyzers o POP-4 for 3100/3100-Avant Genetic Analyzers o POP-4 for 3130/3130xL Genetic Analyzers • POP-7™ Polymer: o POP-7 for 3130/3130xL Genetic Analyzers o POP-7 for 3500/3500xL Genetic Analyzers	402838 4316355 4352755 4352759 4393714	N/A

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 45 µL for each sample + 135 µL (3 x 45 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 50 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 45 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 PCR program in Table 4:

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

Table 4. Thermal cycling conditions

Standard Program			
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

7.4.4. Remove the amplified PCR plate from the thermal cycler.

7.5. Detection

Gel Detection – Heteroduplex Analysis

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

7.5.2. Re-anneal PCR products at 4ºC for 60 minutes.

7.5.3. Assemble electrophoresis unit using a 6% non-denaturing polyacrylamide TBE gel (made with 1X TBE) and 0.5X TBE running buffer.

7.5.4. Add 5 µL of ice-cold non-denaturing bromophenol blue loading buffer to samples.

7.5.5. Load 20 µL of mixture into 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, acrylamide gel thickness and type of PCR equipment.
- Voltage and run time can be adapted accordingly.

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

7.5.8. Destain the gels 2X in water for 5-10 minutes.

7.5.9. Visualize the gel using UV illumination.

7.5.10. Photograph the gel and interpret the data.

ABI Fluorescence Detection with ABI 310, 3100 & 3130 instruments

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

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

7.5.13. 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.

7.5.14. Cap or cover the PCR plate.

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

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

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

7.5.18. Data are automatically displayed as size and color specific peaks. Review profile and controls, report results.

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.

7.5.19. 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.

7.5.20. 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.

7.5.21. 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.

7.5.22. Cap or cover the PCR plate.

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

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

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

7.5.26. Data are automatically displayed as size and color specific peaks. Review profile and controls, report results.

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

7.6. Recommended Positive Controls

The amplicon sizes listed in Table 5 were determined using an ABI platform. The amplicon sizes measured on each specific capillary electrophoresis instrument may differ by 1 to 4 base pairs from those listed depending on the platform of detection and the version of the analysis software used. Once identified, the amplicon size as determined on a specific platform will be consistent from run to run.

Note: "Color" indicates the color of products generated with the master mix when using the default color assignment on ABI fluorescence detection systems.

Table 5. Positive Controls

Master Mix	Target	Color	Control DNA	Catalog #	Product Size (bp)
TCRG - **6FAM**	Vγ1-Vγ11 + Jγ1/Jγ2, JγP, JγP1/JγP2	**Blue**	**Specified Size Range** 5% *TCRG* Positive Control DNA	40883320	**159-207** 194, 196
Specimen Control Size Ladder	Multiple Genes	**Blue**	*TCRG* Negative Control DNA	40920020	84, 96, 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. For ABI fluorescence detection, the 600 bp peak may not appear during normal run times. In addition, the size of this peak may differ by over 30 bp when fragment size is extrapolated using the GeneScan - 400HD [ROX] size standards

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.

8.1. Sample Reporting

Results can be reported as "Positive" or "Negative" for "Detection of 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. If the controls do not yield the correct results, the assay is not valid and the samples cannot be interpreted.

The following describes the analysis for 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 assay.

8.1.3. **Specimen Control Size Ladder**: (This is run on unknown samples only). If the Specimen Control Size Ladder :

- **Generates ~100, 200, 300, 400, and 600 bp products:** Please note smaller PCR fragments are preferentially amplified, and it is not unusual for the 600 bp fragment to have a diminished signal or to be missing entirely. Continue with analysis.
- **Generates no peaks:** Repeat the assay and/or obtain a new specimen.

8.2. Sample Interpretation

Following the acceptance of the controls, the samples are interpreted as follows:

8.2.1. Criteria for defining a positive peak are as follows:

- Products generated from samples that fall within the 159 bp - 207 bp size range and, if using differential fluorescence detection, are at least three times the amplitude of either adjacent peak that might be present in the polyclonal background are consistent with a positive peak.
- One or two prominent peaks within the specified size range (159 bp - 207 bp) are reported as: "**Detection of T-cell receptor gamma gene rearrangement(s) consistent with the presence of a clonal cell population**."

Note: The size of amplified products can vary from instrument to instrument by 1 bp - 4 bp. In addition, valid clonal *TCR* gamma rearrangements can occur outside the specified size range. Product(s) that are suspect *TCR* gamma gene rearrangement(s) that lie outside the specified size range can be sequenced to confirm their identity.

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

Table 6. Expected Size of Amplified Products

Master Mix	Target	Color	Control DNA	Cat#	Product Size (bp)
TCRG – 6FAM	All V and J genes	**Blue**	**Specified Size Range**	---	**159 - 207**
		Blue	*TCRG* Negative Control DNA	40920020	159 - 207
	Vγ9 + Jγ1/Jγ2	**Blue**	5% *TCRG* Positive Control DNA	40883320	194
	Vγ10 + Jγ1/Jγ2	**Blue**	5% *TCRG* Positive Control DNA	40883320	196
Specimen Control Size Ladder	Multiple Genes	**Blue**	Any Human DNA	---	84*, 96, 200, 300, 400, 600

Note: The amplicon sizes listed above were determined using an ABI platform. Amplicon sizes seen on each specific CE instrument may differ 1 bp - 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.

***Note:** An 84 bp peak may be detected and is likely the result of primer-primer interaction.

10.2. Sample Data

The data shown below was generated using the *TCRG* - 6FAM Master Mix.

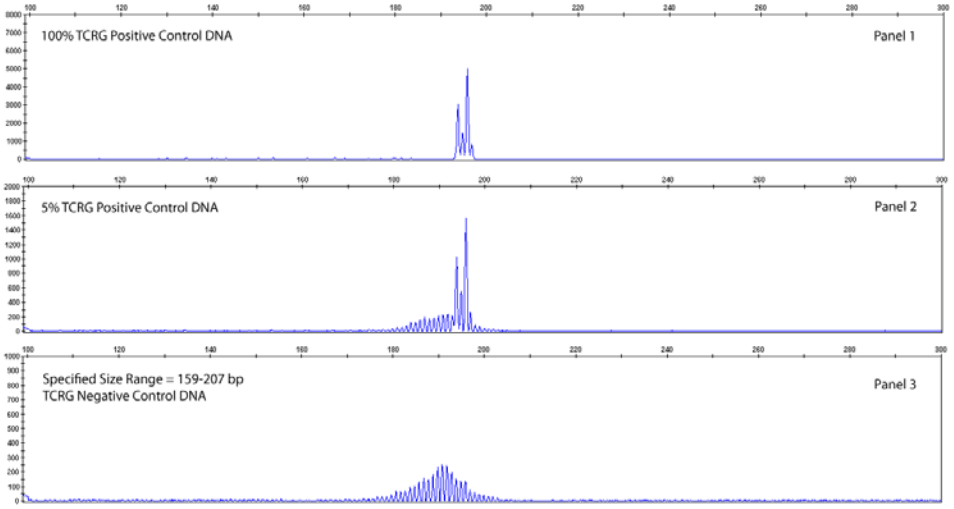

Figure 2. PCR products generated with the *TCRG* – 6FAM Master Mix were run on an ABI instrument.

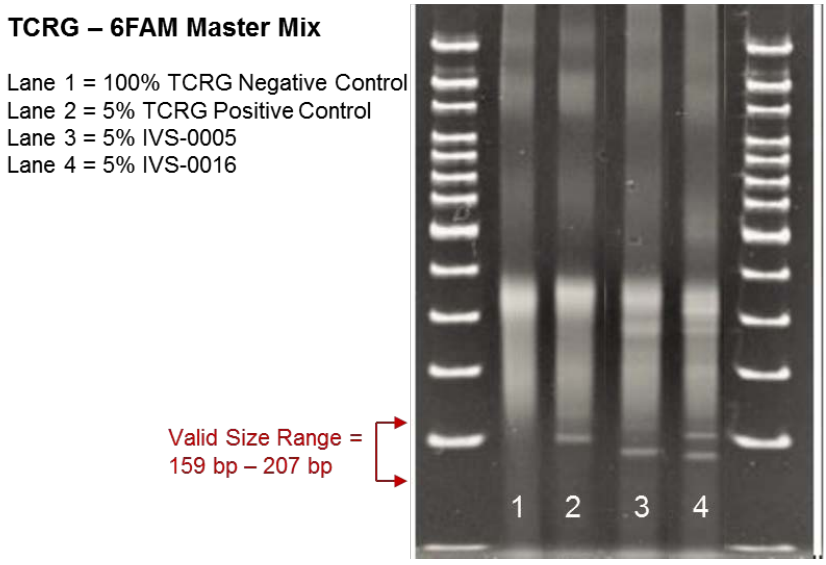

Figure 3. PCR products generated with the *TCRG* – 6FAM Master Mix were heteroduplexed and run on a polyacrylamide gel.

11. Technical and Customer Service

Thank you for purchasing our **T-Cell Receptor Gamma Gene Rearrangement Assay 2.0**. 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.**

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12. References

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13. Symbols

The following symbols are used in labeling for Invivoscribe products.

REF	Catalog Number		Expiration Date
VOL	Reagent Volume	EC REP	Authorized Representative in the European Community
LOT	Lot Number		Consult Instructions for Use
	Storage Conditions	RUO	For Research Use Only

14. Legal Notice

14.1. 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 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 2.0: 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).

15.3. Aliquot volumes are 45 µL for each sample + 135 µL for the positive, negative and no template controls.

15.4. Add an additional 20 µL to correct for pipetting errors.

15.5. Add the appropriate amount of Taq DNA polymerase (0.25 µL @5 U/µL per 50 µL total reaction volume) to each master mix and gently mix by inverting several times or gently vortexing.

15.6. Aliquot 45 µL of master mix to individual wells of a PCR plate.

15.7. 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.

15.8. Amplify target DNA using the universal thermal cycler program.

Gel Detection – Heteroduplex Analysis

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

15.10. Re-anneal PCR products at 4ºC for 60 minutes.

15.11. Assemble electrophoresis unit using a 6% non-denaturing polyacrylamide TBE gel (made with 1X TBE) and 0.5X TBE running buffer.

15.12. Add 5 µL of ice-cold non-denaturing bromophenol blue loading buffer to samples.

15.13. Load 20 µL of mixture into wells of the gel.

15.14. Run gel at 110V for 2-3 hours or 40-50V overnight.

- Voltage and electrophoresis time depend on the PCR amplicon size, acrylamide gel thickness and type of PCR equipment.
- Voltage and run time can be adapted accordingly.

15.15. Stain the gels in 0.5 µg/mL Ethidium Bromide (in water or 0.5X TBE Buffer) for 5-10 minutes.

15.16. Destain the gels 2X in water for 5-10 minutes.

15.17. Visualize the gel using UV illumination.

15.18. Photograph the gel and interpret the data.

ABI Fluorescence Detection with ABI 310, 3100 & 3130 instruments

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

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

15.21. 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.

15.22. Cap or cover the PCR plate.

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

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

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

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

15.26. Review profile and controls, report results.

ABI Fluorescence Detection with ABI 3500 instruments

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

15.28. 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.

15.29. 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.

15.30. Cap or cover the PCR plate.

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

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

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

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

15.34. Review profile and controls, report results.