Instructions for Use

IGL Gene Clonality Assay

RUO

For identification of clonal immunoglobulin lambda light chain gene rearrangements.

RUO 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 11030010	*IGL* Gene Clonality Assay for Gel Detection	33 Reactions
REF 11030011	*IGL* Gene Clonality Assay ABI Fluorescence Detection	33 Reactions
REF 11030021	*IGL* Gene Clonality Assay MegaKit for ABI Fluorescence Detection	330 Reactions

Table of Contents

1. Assay Use

This Research Use Only assay identifies clonal immunoglobulin lambda light chain rearrangements and is useful for the study of:

- Identifying clonal B-cell populations
- Evaluation of new research and methods in malignancy studies.

2. Summary and Explanation of the Test

2.1. Background

Rearrangements of the antigen receptor genes occur during ontogeny in B and T lymphocytes. These gene rearrangements generate products that are unique in length and sequence for each cell. Therefore, polymerase chain reaction (PCR) assays can be used to identify lymphocyte populations derived from a single cell by detecting the unique V-J gene rearrangements present within these antigen receptor loci.[1] This PCR assay employs multiple consensus DNA primers that target conserved genetic regions within the immunoglobulin lambda light chain gene. This test is used to detect the vast majority of clonal B-cell malignancies from DNA. Test products can be analyzed using a variety of detection formats, including gel and capillary electrophoresis.

2.1. Summary

Invivoscribe's gene clonality assays represent a simple approach to PCR-based clonality testing. These standardized assays were carefully optimized testing positive and negative control samples using multiplex master mixes. This test kit includes two (2) master mixes. The *IGL* Tube master mix targets the variable region and the joining region of the immunoglobulin lambda light chain locus. The Specimen Control Size Ladder master mix, targets multiple genes and generates a series of amplicons of 96, 197, 297, 397, and 602 base pairs (bp) 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)

Polymerase chain reaction (PCR) assays are routinely used for the identification of clonal B-cell populations. These tests amplify the DNA between primers that target the variable (V) and joining (J) regions. The 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 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.

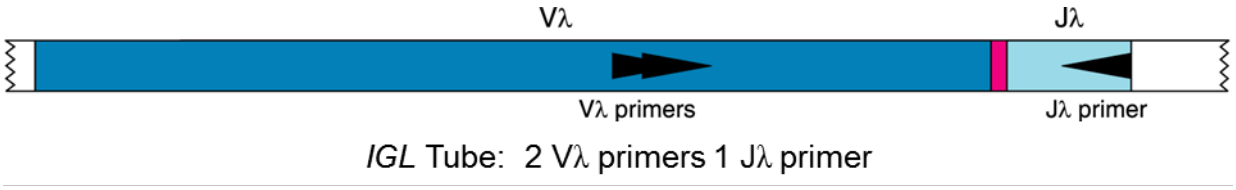

Figure 1. Depicted is a simple representation of the organization of a rearranged immunoglobulin lambda light chain gene on chromosome 22q11.2. Black arrows represent the relative positions of primers. The two Vλ primers target Vλ1, 2, and 3 because these three families cover approximately 70% of rearrangeable Vλ gene segments, and approximately 90% of all *IGL* gene rearrangements involve these three families. Similarly the single Jλ primer only targets Jλ1, 2, and 3 because these three Jλ gene segments are involved in 98% of all *IGL* gene rearrangements.

3.2. Gel Detection

Gel electrophoresis, such as agarose gel electrophoresis or non-denaturing polyacrylamide gel electrophoresisis (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).

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 1 to 2 nucleotides.

4. Reagents

4.1. Reagent Components

Table 1. Available Kits

Catalog #	Description	Quantity
REF 11030010	*IGL* Gene Clonality Assay – Gel Detection	33 Reactions
REF 11030011	*IGL* Gene Clonality Assay – ABI Fluorescence Detection	33 Reactions
REF 11030021	*IGL* Gene Clonality Assay MegaKit – ABI Fluorescence Detection	330 Reactions

Table 2. Reagent Components

Reagent	Catalog #	Reagent Components (active ingredients)	Unit of Quantity	Assay Kit # of Units	MegaKit # of Units	Storage Temp.
Positive Control DNA	40880550	**IVS-0010 Clonal Control DNA** 200 µg/mL of DNA in 1/10th TE	100 µL	1	5	2°C – 8°C or -85°C – -65°C
	40881690	**IVS-0029 Clonal Control DNA** 200 µg/mL of DNA in 1/10th TE	100 µL	1	5	
Negative (Normal) Control DNA	40920010	**IVS-0000 Polyclonal Control DNA** 200 µg/mL of DNA in 1/10th TE	100 µL	1	5	
Gel Detection						
Master Mixes	21030010	***IGL* Tube – Unlabeled** Multiple oligonucleotides targeting the Vλ region and Jλ region of the immunoglobulin lambda light chain gene in a buffered salt solution.	1500 µL	1	N/A	-85°C – -65°C
Amplification Control Master Mix	20960020	**Specimen Control Size Ladder – Unlabeled** Multiple oligonucleotides targeting housekeeping genes.	1500 µL	1	N/A	
ABI Fluorescence Detection						
Master Mixes	21030011	***IGL* Tube – 6FAM** Multiple oligonucleotides targeting the Vλ region and Jλ region of the immunoglobulin lambda light chain gene in a buffered salt solution.	1500 µL	1	10	-85°C – -65°C
Amplification Control Master Mix	20960021	**Specimen Control Size Ladder – 6FAM** Multiple oligonucleotides targeting housekeeping genes.	1500 µL	1	10	

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

4.2. Warnings and Precautions

- **RUO** For RESEARCH USE ONLY. Not for use in diagnostic procedures.
- Use this assay kit as a system. Do not substitute other manufacturer's reagents. Dilution, reducing amplification reaction volumes, or other deviation in this protocol may affect the performance of this test and/or nullify any limited sublicense that comes with the purchase of this testing kit.
- Materials are stable until the labeled expiration date when stored and handled as directed. Do not use kits beyond their expiration date.
- Close adherence to the protocol will assure optimal performance and reproducibility. Ensure 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.
- 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

- Store the assay at -85°C to -65°C until ready to use.
- All reagents and controls must be thawed and vortexed or mixed thoroughly prior to use to ensure that they are resuspended completely.
- 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 (for ABI Detection)

- 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 your polymer and capillary type.
- See section 7.5 *Detection* for further details.

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 standards.

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

6.4. Sample Preparation

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

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 to 400 µg per mL in 1/10th 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.
- 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 ensure sufficient quality and quantity of DNA is present to yield 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 96, 197, 297, 397 and 602 bp. Please note that the 96 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

Please see Table 2 for a list of provided materials.

7.2. Materials Required (Not Provided)

Table 3. Materials Required (not provided)

Reagent/Material	Recommended Reagents/Materials and Suppliers	Catalog Number	Notes
DNA Polymerase	Roche: • EagleTaq DNA Polymerase Invivoscribe, Inc. • FalconTaq DNA Polymerase or equivalent	05206944190 60970130	N/A
Molecular Biology Grade or USP Water	N/A	N/A	Sterile and free of DNase and RNase.
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 (for Agarose or Polyacrylamide Gels)			
Gel Electrophoresis Unit	N/A	N/A	For polyacrylamide gels
Ethidium Bromide	Thermo Fisher Scientific®: • UltraPure® 10 mg/mL Ethidium Bromide	15585-011	N/A
Agarose Gels	Thermo Fisher Scientific: • MetaPhor™ Agarose, 125 g or • Lonza™ NuSieve™ 3:1 Agarose	BMA50180 or BMA50090	N/A
6% Polyacrylamide Gels	Thermo Fisher Scientific: • Novex® TBE Gels (6%, 12 well)	EC62652Box	N/A
TBE Running Buffer	Invitrogen: • 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	Invitrogen: • TrackIt™ 100 bp DNA Ladder	10488-058	N/A
ABI Fluorescence Detection			
ABI Capillary Electrophoresis Instrument	Applied Biosystems: • ABI 310, 3100, 3130 or 3500 series	N/A	N/A
Hi-Di Formamide	Applied Biosystems: • Hi-Di™ Formamide	4311320	N/A

Table 3. Materials Required (not provided)

Reagent/Material	Recommended Reagents/Materials and Suppliers	Catalog Number	Notes
Size Standards	Invivoscribe, Inc.: • Hi-Di Formamide w/ROX size standards for ABI 3100 Applied Biosystems: • For ABI 3100 or 3130 instruments: ○ GeneScan™ - 400HD [ROX]™ • For ABI 3500 instruments: ○ GeneScan - 600 [LIZ]™ v2.0	60980061 402985 4408399	N/A
Spectral Calibration Dye Sets	Applied Biosystems: • For ABI 3100 and 3130 instruments: ○ DS-30 Matrix Standard Kit (Dye Set D) • For ABI 3500 instruments: ○ DS-33 Matrix Standard Kit (Dye Set G5)	4345827 4345833	N/A
Polymer	Applied Biosystems: • POP-4™ Polymer: ○ 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	4316355 4352755 4352759 4393714	N/A
Buffer	Applied Biosystems: • 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 an appropriate volume of each master mix into a sterile microfuge tube (one tube for each master mix).

- Aliquot volumes are 45 µL for each sample + 135 µL (3 x 45 µL) for the positive, negative and no template (water) 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 PCR reaction volume) to the 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 amplification plate from the thermal cycler.

7.5. Detection

- Not all detection formats are available for all assays.

Gel Detection – Agarose TBE Gels

7.5.1. Prepare a 2% MetaPhor or NuSieve 3:1 agarose/TBE gel.
7.5.2. Individually mix 20 µL from each amplification reaction mixed with 4 µL of 6X gel loading buffer.
7.5.3. Load 20 µL of this mixture into separate wells of the gel, flanked by DNA size standards.
7.5.4. Detect products using ethidium bromide or an equivalent dye.
7.5.5. Photgraph the gel and interpret the data.

Gel Detection – Polyacrylamide TBE Gels

7.5.6. Assemble electrophoresis unit using a 6% non-denaturing polyacrylamide TBE gel (made with 1X TBE) and 0.5X TBE running buffer.
7.5.7. Add 5 µL of ice-cold non-denaturing bromophenol blue loading buffer to samples.
7.5.8. Load 20 µL of mixture into wells of the gel.
7.5.9. 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.

7.5.10. Stain gels in 0.5 µg/mL ethidium bromide (in water or 0.5X TBE Buffer) for 5-10 minutes.
7.5.11. Destain gels 2X in water for 5-10 minutes.
7.5.12. Visualize the gel using UV illumination.
7.5.13. Photograph the gel and interpret the data.

Gel Detection – Heteroduplex Analysis (RECOMMENDED)

7.5.14. Denature 20 µL of PCR products at 94ºC for 5 minutes.
7.5.15. Re-anneal PCR products at 4ºC for 60 minutes.
7.5.16. Assemble electrophoresis unit using a 6% non-denaturing polyacrylamide TBE gel (made with 1X TBE) and 0.5X TBE running buffer.
7.5.17. Add 5 µL ice-cold non-denaturing bromophenol blue loading buffer to samples.
7.5.18. Load 20 µL of mixture into wells of the gel.
7.5.19. 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.

7.5.20. Stain gels in 0.5 µg/mL ethidium bromide (in water or 0.5X TBE Buffer) for 5-10 minutes.
7.5.21. Destain gels 2X in water for 5-10 minutes.
7.5.22. Visualize the gel using UV illumination.
7.5.23. Photograph the gel and interpret the data.

ABI Fluorescence Detection with ABI 310, 3100, & 3130 instruments

7.5.24. In a new microcentrifuge tube, mix an appropriate amount (10 µL per reaction) of Hi-Di Formamide with ROX Size Standards[a]. Vortex well.
7.5.25. In a new 96-well PCR plate, add 10 µL of Hi-Di Formamide with ROX size standards to individual wells for each reaction.
7.5.26. 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.
7.5.27. Cap or cover the PCR plate.
7.5.28. Heat denature the samples at 95ºC for 2 minutes, then snap chill on ice for 5 minutes.
7.5.29. Prepare a **sample sheet** and **injection list** for the samples.
7.5.30. Run the samples on an ABI 310/3100/3130 capillary electrophoresis instrument according to its user manual.
7.5.31. Data are automatically displayed as size and color specific peaks. Review profile and controls.

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.

7.5.32. 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.
7.5.33. In a new 96-well PCR plate, add 9.5 µL Hi-Di Formamide with LIZ size standards to individual wells for each reaction.
7.5.34. 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.
7.5.35. Cap or cover the PCR plate.
7.5.36. Heat denature the samples at 95ºC for 3 minutes, then snap chill on ice for 5 minutes.
7.5.37. Prepare a **sample sheet** and **injection list** for the samples.
7.5.38. Run the samples on an ABI 3500 capillary electrophoresis instrument according to its user manual.
7.5.39. Data are automatically displayed as size and color specific peaks. Review profile and controls.

[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

Table 5: Recommended Positive Control Amplified Product Sizes

Master Mix	Target	Color	Control DNA	Catalog #	Product Size (bp)
***IGL* Tube**	Vλ-Jλ	**Blue**	**Valid Size Range** IVS-0010 Clonal Control DNA IVS-0029 Clonal Control DNA	--- 40880550 40881690	**135 - 170** 139 143, 156
Specimen Control Size Ladder	Multiple Genes	**Blue**	**Valid Size Range** IVS-0000 Polyclonal Control DNA	--- 40920010	**96, 197, 297, 397, 602** 96, 197, 297, 397, 602

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 each specific isntrumentwill be consistent from run to run.

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 Next Generation Sequencing (NGS) may be advisable to rule out clonality.

8.1. Analysis

The size range for each master mix has been determined testing positive control samples. 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.

Results can be reported as "Positive" or "Negative" for "Detection of clonal immunoglobulin lambda light 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 of each of the controls 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.
- **Specimen Control Size Ladder**: (This is run on unknown samples only) If the Specimen Control Size Ladder:
  - **Generates 96, 197, 297, 397 and 602 bp products:** Continue with analysis. Smaller PCR fragments are preferentially amplified, it is not unusual for the 602 bp fragment to have a diminished signal or to be missing entirely.
  - **Does not generate specified product:** Repeat 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 immunoglobulin lambda light 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 Values

10.1. Expected Size of Amplified Products

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

Table 6: Expected Size of Amplified Products

Master Mix	Target	Color	Control DNA	Catalog #	Product Size in bp
***IGL* Tube**	Vλ-Jλ	**Blue**	**Valid Size Range** IVS-0000 Polyclonal Control DNA IVS-0010 Clonal Control DNA IVS-0029 Clonal Control DNA	--- 40920010 40880550 40881690	**135 - 170** 135 - 170 127[1], 139 126[1], 143, 156
Specimen Control Size Ladder	Multiple Genes	**Blue**	Any Human DNA	---	**96, 197, 297, 397, 602** 96, 197, 297, 397, 602

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 your specific platform will be consistent from run to run.

[1]**Note:** Amplicon product is often not observed.

10.2. Sample Data

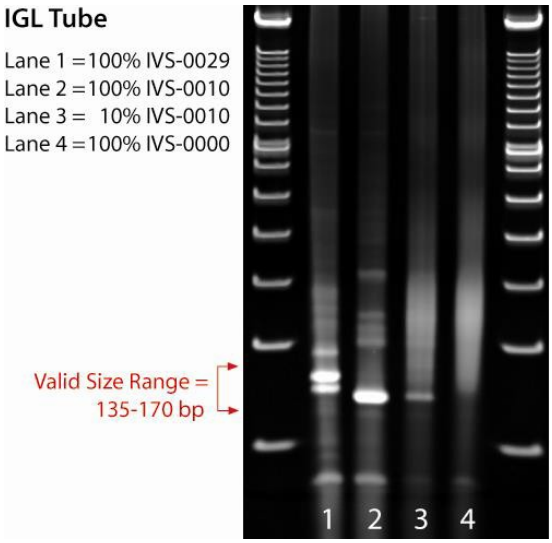

Figure 2. Gel Detection.
The image at left was generated using the *IGL* master mix and PCR product was run on a 6% polyacrylamide gel.

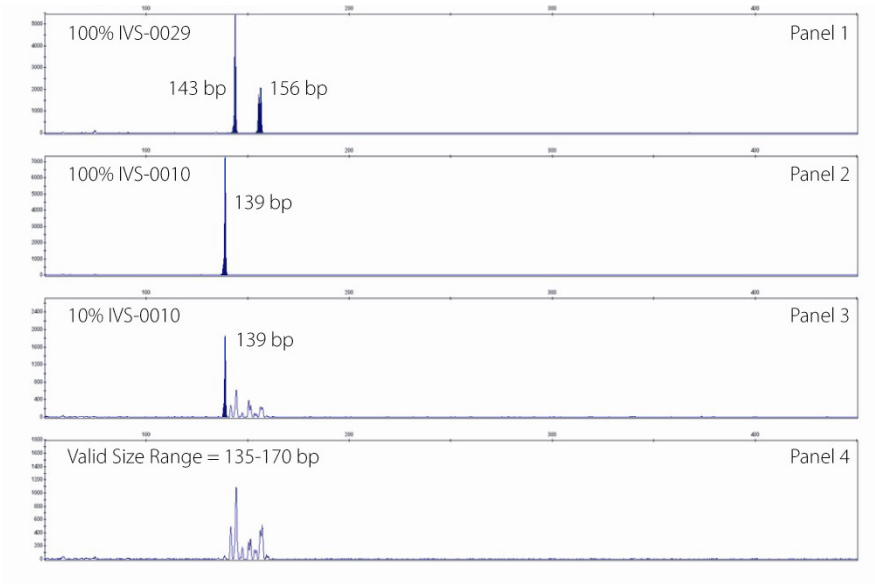

Figure 3. ABI Fluorescence Detection.
The image at right was generated using the *IGL* Tube – 6FAM master mix with an ABI instrument.

11. Technical and Customer Service

Thank you for purchasing our ***IGL*** **Gene Clonality 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

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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 labeling for Invivoscribe products.

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

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. *IGL* Gene Clonality 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 a sterile microfuge tube (one tube for each master mix).

- Aliquot volumes are 45 µL for each sample + 135 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 50 µL total PCR reaction volume) to each master mix and gently mix by inverting several times or gently vortexing.

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

15.5. Add 5 µL 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.

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

Gel Detection – Heteroduplex Analysis

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

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

15.9. Assemble electrophoresis unit using a 6% non-denaturing polyacrylamide TBE gel and 0.5X TBE running buffer.

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

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

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

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

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

15.15. Visualize gel using UV illumination.

15.16. Photograph gel and interpret the data.

ABI Fluorescence Detection with ABI 310, 3100, & 3130 instruments

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

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

15.19. 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.

15.20. Cap or cover the PCR plate.

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

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

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

15.24. Review profile and controls.

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.

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

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

15.27. 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.

15.28. Cap or cover the PCR plate.

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

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

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

15.32. Review profile and controls.