IdentiClone®

Instructions for Use

IdentiClone® *IGL* Gene Clonality Assay

CE UK CA IVD

For Identification of Clonal Immunoglobulin Lambda Light Chain gene rearrangements.

IVD For *In Vitro* Diagnostic Use

invivoscribe

Storage Conditions: -85°C to -65ºC

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

Catalog#	Products	Quantity
REF 91030011	IdentiClone *IGL* Gene Clonality Assay – ABI Fluorescence Detection	33 Reactions
REF 91030021	IdentiClone *IGL* Gene Clonality Assay MegaKit – ABI Fluorescence Detection	330 Reactions

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1. Intended Use

The IdentiClone *IGL* Gene Clonality Assay is an *in vitro* diagnostic product intended for PCR-based detection of clonal immunoglobulin lambda light chain gene rearrangements in patients with suspect lymphoproliferations. Specifically, the *IGL* Gene Clonality Assay can be used to:

- Identify clonality in atypical lymphoproliferative disorders
- Support a differential diagnosis between reactive lesions and hematologic malignancies
- Assign presumptive lineage in mature monoclonal lymphoproliferative disorders
- Identify tumor-specific markers (*IGL* gene rearrangements) for post-treatment monitoring
- Monitor and evaluate disease recurrence

2. Summary and Explanation of the Test

2.1. Background

Rearrangements of the antigen receptor genes occur during ontogeny in B and T lymphocytes. These gene rearrangements generate products that are unique in length and sequence for each cell. Therefore, polymerase chain reaction (PCR) assays can be used to identify lymphocyte populations derived from a single cell by detecting the unique V-J gene rearrangements present within these antigen receptor loci.[1] This 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.

Invivoscribe's IdentiClone assays represent a new approach to PCR-based clonality testing. These standardized assays were carefully optimized testing positive and negative control samples using multiplex master mixes. Assay development was followed by extensive validation including the testing of more than 400 clinical samples using Revised European/American Lymphoma (REAL) Classification. Testing was performed at more than thirty prominent independent testing centers throughout Europe in a collaborative study known as the BIOMED-2 Concerted Action.[2]

The ABI detection based assays cannot reliably detect clonal populations comprising less than 1% of the total lymphocyte cell population. **Always interpret the results of molecular clonality tests in the context of clinical, histological and immunophenotypic data**.

2.2. Summary

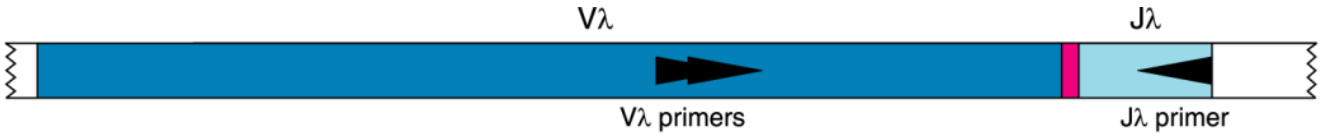
This test kit includes two (2) master mixes. The *IGL* Tube master mix targets the variable region and the joining region of the immunoglobulin lambda light chain locus. The Specimen Control Size Ladder master mix, targets multiple genes and generates a series of amplicons of approximately 96, 197, 297, 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)

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



***IGL* Tube: 2λ V primers + 1 Jλ primer**

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.

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

3.2. 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. This reproducibility and sensitivity coupled with the automatic archiving of specimen data allows for the monitoring, tracking, and comparison of data from individual patients over time.

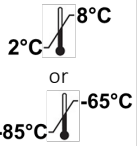
4. Reagents

4.1. Reagent Components

Table 1. Available Kits

Catalog #	Description	Quantity
REF 91030011	IdentiClone *IGL* Gene Clonality Assay – ABI Fluorescence Detection	33 Reactions
REF 91030021	IdentiClone *IGL* Gene Clonality Assay MegaKit – ABI Fluorescence Detection	330 Reactions

Table 2. Reagent Components

Reagent	Catalog #	Reagent Components (active ingredients)	Unit of Quantity	91030011 # of Units	91030021 # of Units	Storage Temp.
Master Mixes	REF 21030011CE	***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	
Template Amplification Master Mix	REF 20960021	**Specimen Control Size Ladder – 6FAM** Multiple oligonucleotides targeting housekeeping genes.	1500 µL	1	10	
Positive Control DNA	REF 40880550	**IVS-0010 Clonal Control DNA** 200 µg/mL of DNA in 1/10th TE	100 µL	1	5	
	REF 40881690	**IVS-0029 Clonal Control DNA** 200 µg/mL of DNA in 1/10th TE	100 µL	1	5	
Negative (Normal) Control DNA	REF 40920010	**IVS-0000 Polyclonal Control DNA** 200 µg/mL of DNA in 1/10th TE	100 µL	1	5	

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

4.2. Warnings and Precautions

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

4.3. Storage and Handling

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

5. Instruments

5.1. Thermal cycler

- Use or Function: Amplification of DNA samples
- 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 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 But Not Provided.*
- Use the default settings for your polymer and capillary type.
- See section *7.5 ABI Fluorescence Detection* 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 the OSHA Standard on Bloodborne Pathogens or Biosafety Level 2.

6.2. Interfering Substances

The following substances are known to interfere with PCR:

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

6.3. Specimen Requirements and Handling

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

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

6.4. Sample Preparation

Extract the genomic DNA from patient specimens as soon as possible. Resuspend DNA to a final concentration of 100 µg to 400 µg per mL in 1/10th 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. Testing sample DNA with the Specimen Control Size Ladder master mix will ensure that DNA of sufficient quality and quantity was present to yield a valid result.

6.5. Sample Storage

Store genomic DNA(gDNA) at 2°C to 8°C or long term at -85°C to -65°C.

7. Assay Procedure

7.1. Materials Provided

Table 3: Kit components

Catalog #	Description
REF 21030011CE	*IGL* Tube – 6FAM
REF 20960021	Specimen Control Size Ladder – 6FAM
REF 40880550	IVS-0010 Clonal Control DNA
REF 40881690	IVS-0029 Clonal Control DNA
REF 40920010	IVS-0000 Polyclonal Control DNA

7.2. Materials Required But Not Provided

Table 4: Materials Required (not provided)

Reagent/Material	Recommended Reagents/Materials and Suppliers	Catalog # (REF)	Notes
DNA Polymerase	Roche: • EagleTaq DNA Polymerase Invivoscribe, Inc.: • FalconTaq DNA Polymerase or equivalent	05206944190 60970130	N/A
Glass Distilled De-ionized Molecular Biology Grade or USP Water	N/A	N/A	Sterile and free of DNase and RNase.
Calibrated Pipettes	Rainin: • P-2, P-20, P-200, and P-1000 pipettes • Or SL-2, SL-20, SL-200, and SL-1000 pipettes	N/A	Must be able to accurately measure volumes between 1 µL and 1000 µL.
Thermal cycler	Thermo Fisher Scientific: • Veriti Dx Thermal Cycler Bio-Rad: • 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
ABI Capillary Electrophoresis Instrument	Applied Biosystems: • ABI 310, 3100, or 3500 series	S.O.	N/A
Hi-Di Formamide	Applied Biosystems: • Hi-Di™ Formamide	4311320	N/A
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)	4345827	N/A

Table 4: Materials Required (not provided)

Reagent/Material	Recommended Reagents/Materials and Suppliers	Catalog # (REF)	Notes
Spectral Calibration Dye Sets	• 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)	402996 401546 4345833	N/A
Polymer	Applied Biosystems: • 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
Buffer	Applied Biosystems: • 10X Genetic Analyzer Buffer with EDTA	402824	Dilute 1:10 in sterile water before use

7.3. Reagent Preparation

- All unknown samples can be tested using the Specimen Control Size Ladder master mixto ensure that no inhibitors of amplification are present and there is DNA of sufficient quality and quantity to generate a valid result.
- Singlicate test results are valid; however, when possible, test in **duplicate**. If duplicate testing provides inconsistent results, re-testing or re-evaluation of the sample is necessary.
- **Test positive**, **negative** and **no template** controls for each master mix.

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

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

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

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

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

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

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

7.3.3. Add 1.25 U (or 0.25 µL @5 U/µL) of Taq DNA polymerase per reaction to each master mix.

- The total Taq DNA polymerase added to each master mix = **n x 0.25 µL**, and **m x .25 µL** for the Specimen Control Size Ladder master mix.
- Gently vortex to mix.

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

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

7.3.6. Cap or cover the PCR plate.

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

Quick Guide:

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

n × 45 µL Master Mix

n × 0.25 µL Taq DNA polymerase

Vortex gently to mix.

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

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

Total reaction volume = **50 µL**

7.4. Amplification

7.4.1. Amplify the samples using the following PCR program:

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

Table 5: Thermal cycling conditions

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

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

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

7.5. ABI Fluorescence Detection

- Please note that for ABI fluorescence detection a preceding peak is often observed and is an artifact due to the detection method the ABI platforms use. Preceding peaks are sometimes skewed and have bases that slope on the right side towards the real peak. This is especially evident in the Specimen Control Size Ladder master mix where the 96- nucleotide peak has a preceding peak that shows up at 84 nucleotides.
- Do not multiplex PCR products from different master mixes, as this will result in overall reduced sensitivity of the assay.

ABI 310, 3100, OR 3130 Platforms

7.5.1. In a new microcentrifuge tube, mix an appropriate amount (for a total of 10 µL per reaction) of Hi-Di Formamide with ROX Size Standards. 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 PCR 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 or tubes.

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 capillary electrophoresis instrument according to the user manual.

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

7.5.8. Review profile and controls, report results. (See sections 8*: Interpretation of Results* and 10*: Expected Values*.)

ABI 3500 Platforms:

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. 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 the user manual.

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

7.5.16. Review profile and controls, report results. (See sections 8*: Interpretation of Results* and 10*: Expected Values*.)

7.6. Quality Control

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

7.7. Recommended Positive Controls

The amplicon sizes listed were determined using an ABI platform. Amplicon sizes observed on each specific capillary electrophoresis instrument may differ 1 to 4 nucleotides (nt) 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 your specific platform will be consistent from run to run. This reproducibility is extremely useful when monitoring disease recurrence.

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: Recommended Positive Controls

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

[a]Note: A weak preceding peak around 127 nt may be observed.
[b]Note: Because smaller PCR fragments are preferentially amplified, it is not unusual for the 602 nt fragment to have a diminished signal or to be missing entirely. For ABI fluorescence detection the 602 nt peak may not appear during normal run times. In addition, the size of this peak may differ by over 30 nt when fragment size is extrapolated using the GeneScan - 400HD [ROX] size standards.

8. Interpretation of Results

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

8.1. Analysis

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

Table 7: The following describes the analysis of each control, and the decisions necessary based upon the results.

Type of Control	Expected Result	Aberrant Result
No Template Control	No amplification present, continue with analysis	Amplification present, Repeat the assay.
Polyclonal Control	Product size is consistent with expected size listed in section *10.1 Expected Size of Amplified Products*. No clonal rearrangements are present. Continue with analysis.	Clonal rearrangements are present. Repeat the assay
Positive Control (This can also be an extraction control if positive control material is taken through extraction processes)	Product size is consistent with expected size listed in section *10.1 Expected Size of Amplified Products*. Continue with analysis.	Repeat the assay.
Specimen Control Size Ladder (This amplification control is essential for samples of unknown quantity and quality.)	If all of the 96, 197, 297, 397 and 602 nt peaks are observed, continue with analysis. Because smaller PCR fragments are preferentially amplified, it is not unusual for the 602 nt fragment to have a diminished signal or to be missing entirely. Continue with analysis.	If no bands are observed, repeat the assay unless specimen tests positive. If only 1, 2, or 3 bands are observed, re-evaluate sample for DNA degradation unless specimen tests positive.

8.2. Sample Interpretation

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

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

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

- An absence of positive peaks[a] within the valid size range is reported as:

  "**Negative for the detection of clonal immunoglobulin lambda light chain gene rearrangement(s)**."

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

- Products generated from **diagnostic samples** that fall within the valid size range and are at least three times the amplitude of the third largest peak in the polyclonal background are consistent with a positive peak.
- Products generated from **samples collected after initial diagnosis** that fall within the valid size range and are either; 1) at least three times the amplitude of the third largest peak; or, 2) exceed the amplitude of adjacent neighboring peaks and are identical in size to clonal amplicon products previously generated from the same patient using the same master mix, are consistent with a positive peak.

9. Limitations of Procedure

- This assay does not identify 100% of clonal cell populations.
- This assay cannot reliably detect less than one (1) positive cell per 100 normal cells.
- Always interpret the results of molecular clonality tests in the context of clinical, histological and immunophenotypic data.
- PCR-based assays are subject to interference by degradation of DNA or to inhibition of PCR due to EDTA, heparin, and other agents.

10. Expected Values

10.1. Expected Size of Amplified Products

The amplicon sizes listed were determined using an ABI platform. Amplicon sizes seen on your specific capillary electrophoresis instrument may differ 1 to 4 nucleotides (nt) 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 your specific platform will be consistent from run to run. This reproducibility is extremely useful when monitoring disease recurrence.

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

Table 8: Expected Size of Amplified Products

Master Mix	Target	Color	Control DNA	Catalog #	Product Size in Nucleotides (nt)
***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 - 170 139[a] 143[a], 156
Specimen Control Size Ladder	Multiple Genes	Blue	**Valid Size Range** IVS-0000 Polyclonal Control DNA	--- 40920010	**96, 197, 297, 397, 602[b]** 96, 197, 297, 397, 602[b]

[a]Note: A weak preceding peak around 127 nt may be observed.

[b]Note: Because smaller PCR fragments are preferentially amplified, it is not unusual for the 602 nt fragment to have a diminished signal or to be missing entirely. For ABI fluorescence detection the 602 nt peak may not appear during normal run times. In addition, the size of this peak may differ by over 30 nt when fragment size is extrapolated using the GeneScan - 400HD [ROX] size standards.

10.2. Sample Data

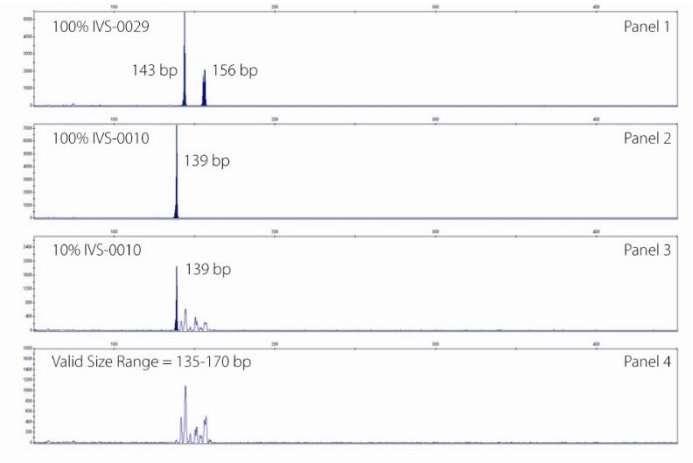


Figure 2. The data shown at left were generated using the *IGL* Tube master mix.

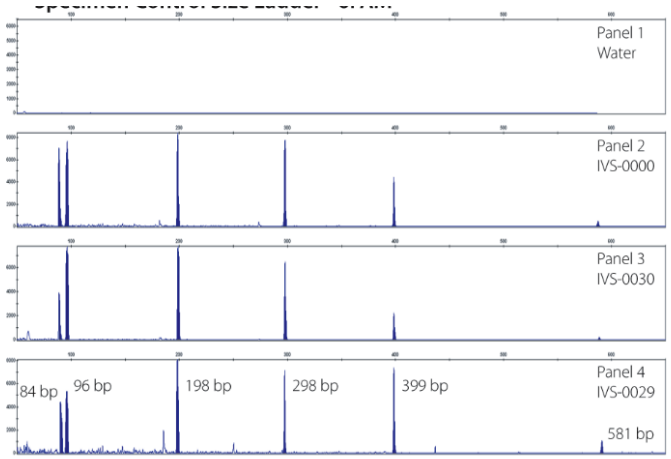


Figure 3. The data shown at left were generated using the Specimen control Size Ladder master mix.

11. Performance Characteristics

This IdentiClone *IGL* Gene Clonality PCR test is a rapid and reliable procedure that is far more sensitive than Southern Blot (SB) analysis in detecting clonality in suspect lymphoproliferations. The final clinico-histopathological diagnosis correlates well with PCR results in a higher number of patients in comparison with SB results.[2]

Table 9. Concordance Studies

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

Table 10. PCR vs. SB analysis relative to histopathology and final diagnosis

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

The diagnostic accuracy of this IdentiClone test was determined to be at least 89%. There were no clear false positive results generated using the IdentiClone tests and there was a high level of precision. In addition a clear benefit of this assay was that clonal results generated allowed for subsequent detection of patient- and tumor-specific gene rearrangements for minimal residual disease detection.

12. Bibliography

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.

13. Technical and Customer Service

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14. Symbols

The following symbols are used in labeling for Invivoscribe diagnostic 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	IVD	For *In Vitro* Diagnostic Use
UDI	Unique Device Identifier		Manufacturer
UK CA	UK Conformity Assessed	UKRP	UK Responsible Person
CH REP	Swiss Authorized Representative	CE	European Conformity

15. Legal Notice

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

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