IdentiClone®

Instructions for Use

CE IVD

IdentiClone® *IGL* Gene Clonality Assay

For Identification of Clonal Immunoglobulin Lambda Light Chain (*IGL*) gene rearrangements.

IVD For *In Vitro* Diagnostic Use

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 91030010	IdentiClone *IGL* Gene Clonality Assay – Gel Detection	33 Reactions

Table of Contents

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 and 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 (*IGL*) 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 gel detection based assays cannot reliably detect clonal populations comprising less than 5% of the total lymphocyte cell population. **Always interpret the results of molecular clonality tests in the context of clinical, histological and immunophenotypic data**.

2.2. Summary

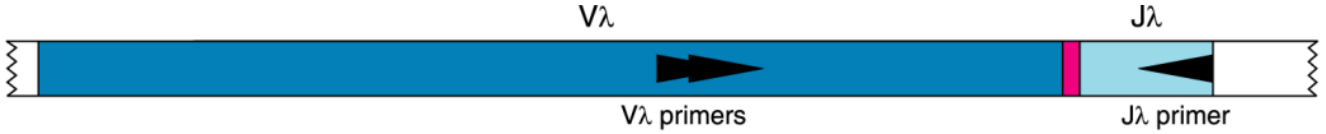
This test kit includes two (2) master mixes. The *IGL* Tube master mix targets the variable (V) and joining (J) regions of the immunoglobulin lambda light chain locus. The Specimen Control Size Ladder master mix targets multiple genes and generates a series of amplicons of approximately 100, 200, 300, 400, and 600 base pairs to ensure that the quality and quantity of input DNA is adequate to yield a valid result. A single thermal cycler program and similar detection methodologies are used with all of our Gene Clonality Assays which improves consistency and facilitates cross training on a broad range of different assays.

This assay is based on 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) and conserved joining (J) regions (*IGL* Tube master mix). 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. 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: Two (2) Vλ primers + one (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. 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, causing a separation of 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 and run normally through the polyacrylamide gel. Therefore, heteroduplex analysis of a clonal sample with a polyclonal background will cause most of the polyclonal product to run much slower through the polyacrylamide gel, thereby increasing their separation and the ability to identify the clonal band(s).

4. Reagents

4.1. Reagent Components

Table 1: Available Assays

Catalog #	Description	Quantity
REF 91030010	IdentiClone *IGL* Gene Clonality Assay – Gel Detection	33 Reactions

Reagent	Catalog # (REF)	Reagent Components (active ingredients)	Unit Quantity	91030010 # of Units	Storage Temp.
Master Mixes	21030010CE	***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	-85°C to -65°C
Template Amplification Control Master Mix	20960020	**Specimen Control Size Ladder – Unlabeled** Multiple oligonucleotides targeting housekeeping genes.	1500 µL	1	
Positive Control DNAs	40880550	**IVS-0010 Clonal Control DNA** 200 µg/mL of DNA in 1/10th TE solution	100 µL	1	2°C to 8°C or -85°C to -65°C
	40881690	**IVS-0029 Clonal Control DNA** 200 µg/mL of DNA in 1/10th TE solution	100 µL	1	
Negative (Normal) Control DNA	40920010	**IVS-0000 Polyclonal Control DNA** 200 µg/mL of DNA in 1/10th TE solution	100 µL	1	

4.2. Warnings and Precautions

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

4.3. Storage and Handling

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

5. Instruments

5.1. Thermal cycler

- Use or function: Amplification of DNA samples
- Performance characteristics and specification:
  - Minimum Thermal Range: 15°C to 96°C
  - Minimum Ramping Speed: 0.8°C/sec
- Follow manufacturer's installation, operation, calibration, and maintenance procedures.
- See section 7.4: *Amplification* for thermal cycler program.

5.2. Electrophoresis Unit

- Use or function: DNA fragment separation
- Performance characteristics and specification:
  - Capable of running at 35V to 135V for extended times
- Follow manufacturer's installation, operation, calibration, and maintenance procedures.

5.3. UV Illumination Unit

- Use or function: DNA detection
- Performance characteristics and specification:
  - Capable of emitting light at a wavelength of ~302 nm
- Follow manufacturer's installation, operation, calibration, and maintenance procedures.

6. Specimen Collection and Preparation

6.1. Precautions

Biological specimens from humans may contain potentially infectious materials. Handle all specimens in accordance with the OSHA Standard on Bloodborne Pathogens or Biosafety Level 2.

6.2. Interfering Substances

The following substances are known to interfere with PCR:

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

6.3. Specimen Requirements and Handling

This assay tests **genomic DNA** (gDNA) from the following sources:

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

6.4. Sample Preparation

Extract the genomic DNA from patient specimens as soon as possible. Resuspend DNA to a final concentration of 100 µg to 400 µg per mL in $1/10^{th}$ 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 is present to yield a valid result.

6.5. Sample Storage

Store gDNA at 2°C to 8°C or at -85°C to -65°C for long term storage.

7. Assay Procedure

7.1. Materials Provided

Table 2: Kit components

Catalog #	Description
REF 21030010CE	*IGL* Tube – Unlabeled
REF 20960020	Specimen Control Size Ladder – Unlabeled
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 (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.: • EagleTaq DNA Polymerase[1] or equivalent	05206944190 60970100	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	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 Electrophoresis Unit	N/A	N/A	For polyacrylamide gels
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

[1]**Note:** This product is for sale and use in the European Economic Area only. It is not to be resold or transferred to another party. See also Legal Notice in section 15.

7.3. Reagent Preparation

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

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

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

- Aliquot volumes are 45 µL for each reaction.
- Include an additional reaction volume 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 is **n × 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 is **m × 45 µL**.

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

- The total Taq DNA polymerase added to each master mix is **n × 0.25 µL**, and **m × 0.25 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 4: Thermal cycling conditions

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

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

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

7.5. Gel Detection – Heteroduplex Analysis

- Do not heteroduplex the PCR products from the Specimen Control Size Ladder master mix. Skip steps 7.5.1 – 7.5.2 and proceed with step 7.5.3.

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

7.5.2. Quick chill to re-anneal PCR products at 4ºC (on an ice water bath) for 60 minutes.

7.5.3. Assemble the electrophoresis unit using a non-denaturing, 6% polyacrylamide TBE gel and 1X TBE running buffer.

7.5.4. Mix 20 µL of each sample with 5 µL of ice-cold non-denaturing 5X bromophenol blue loading buffer.

7.5.5. Load all 20 µL of the mixture into individual wells of the gel.

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

- Voltage and electrophoresis time depend on the PCR amplicon size and polyacrylamide gel thickness.
- Voltage and run time can be adapted accordingly.

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

7.5.8. De-stain gels in water for 5-10 minutes. Repeat with fresh water.

7.5.9. Place gel over UV illuminator to visualize bands

7.5.10. Photograph and interpret resulting data. (See sections 8: *Interpretation of Results* and 10: *Expected Values*)

7.6. Quality Control

Positive and negative (or normal) controls are furnished with the kit and can be run in singlicate each time the assay is performed to ensure proper performance of the assay. In addition, include a no template control (*e.g.,* water) to test for contamination of the master mix or cross-contamination of reactions. A buffer control may also be added to ensure that no contamination of the buffer used to resuspend the samples has occurred. The values for the positive controls are provided 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.

Table 5: Recommended Positive Controls

Master Mix	Target	Control DNA	Catalog #	Product Size in base pairs (bp)
***IGL* Tube**	Vλ - Jλ	**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	**Valid Size Range** IVS-0000 Polyclonal Control DNA	--- 40920010	**100, 200, 300, 400, 600[a]** 100, 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.

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 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 6: The following describes the analysis of each of the controls, and the decisions necessary based upon the results.

Type of Control	Expected Result	Aberrant Result
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 100, 200, 300, 400, and 600 bp peaks are seen, continue with analysis. Because smaller PCR fragments are preferentially amplified, it is not unusual for the 600 bp fragment to have a diminished signal or to be missing entirely. Continue with analysis.	If no bands are seen, repeat the assay unless specimen tests positive. If only 1, 2, or 3 bands are seen, 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:

8.2.1. One or two prominent positive bands[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."**

8.2.2. An absence of positive bands[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 band are as follows:

- Products generated from samples that fall within the valid size range and produce a discrete band(s) distinct from any background smear are consistent with a positive band.

9. Limitations of Procedure

- This assay does not identify 100% of clonal cell populations.
- This assay cannot reliably detect less than five (5) positive cells per 100 normal cells.
- Always interpret the results of molecular clonality tests in the context of clinical, histological and immunophenotypic data.
- PCR-based assays are subject to interference by degradation of DNA or to inhibition of PCR due to EDTA, heparin, and other agents.

10. Expected Values

10.1. Expected Size of Amplified Products

- The amplicon sizes listed were determined using an ABI platform.

Table 7: Expected Sizes of Amplified Products

Master Mix	Target	Control DNA	Catalog #	Product Size in base pairs (bp)
***IGL* Tube**	Vλ - Jλ	**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	**Valid Size Range** IVS-0000 Polyclonal Control DNA	--- 40920010	**100, 200, 300, 400, 600[a]** 100, 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.

10.2. Sample Data

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

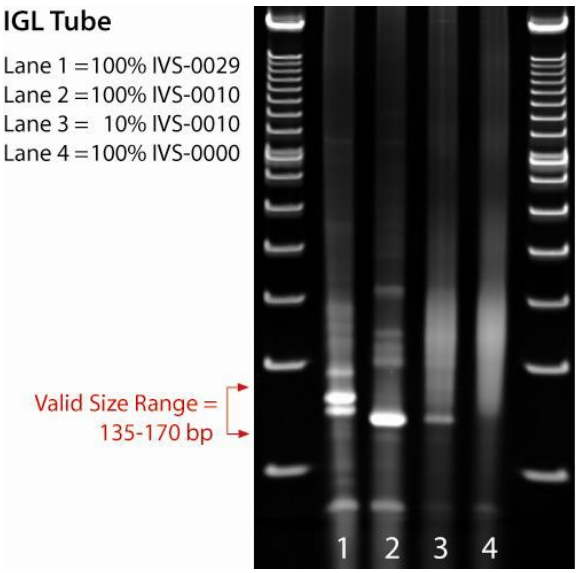


Figure 2. *IGL* Tube master mix.

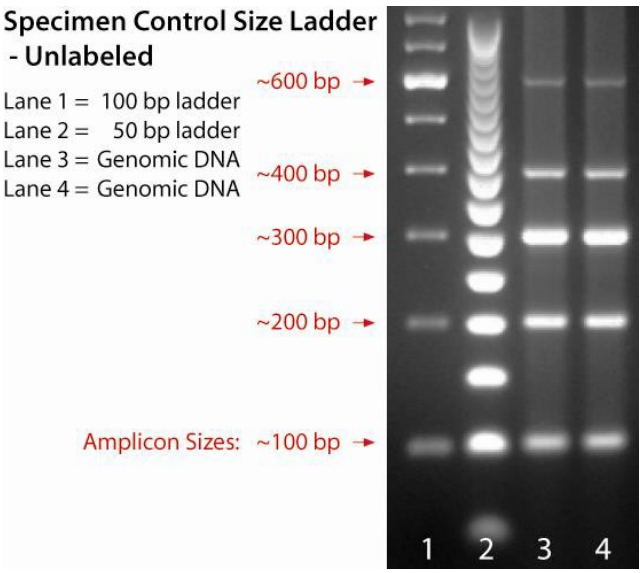


Figure 3. Specimen Control Size Ladder master mix.

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 8. Concordance Studies

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

The diagnostic accuracy of this IdentiClone assay was determined to be at least 89%. There were no clear false positive results generated using the IdentiClone assays and there was a high level of precision. Consequentially, a clear benefit of this assay is that results generated for clonality allow 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

Technical and Customer Service Representatives are available Monday through Friday to answer phone, e-mail, or website inquiries.

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14. Symbols

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

IVD	For *In Vitro* Diagnostic Use		Expiration Date
REF	Catalog Number	EC REP	Authorized Representative in the European Community
VOL	Reagent Volume		Manufacturer
LOT	Lot Number		Consult Instructions for Use
	Storage Conditions		

15. Legal Notice

15.1. Warranty and Liability

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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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