IdentiClone®

Instructions for Use

CE IVD

IdentiClone® *IGH* + *IGK* B-Cell Clonality Assay

For identification of clonal immunoglobulin heavy chain and kappa light chain gene rearrangements.

IVD For *In Vitro* Diagnostic Use

invivoscribe

Storage Conditions: **-85°C to -65°C**
(DNA controls may be separated from assay kits and stored at 2°C to 8°C)

Catalog#	Products	Quantity
REF 91000010	IdentiClone *IGH* + *IGK* B-Cell Clonality Assay – Gel Detection	33 Reactions

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1. Intended Use

The IdentiClone *IGH* + *IGK* B-Cell Clonality Assay is an *in vitro* diagnostic product intended for PCR-based detection of clonal immunoglobulin heavy chain and kappa light chain gene rearrangements in patients with suspect lymphoproliferations. Specifically, the *IGH* + *IGK* B-Cell Clonality Assay can be used to:

- Identify clonality in atypical lymphoproliferative disorders
- Support a differential diagnosis between reactive lesions and hematologic malignancies[4]
- Assign presumptive lineage in mature monoclonal lymphoproliferative disorders
- Identify tumor-specific markers (*IGH* and *IGK* 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 heavy chain and kappa light chain genes. 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 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 testing of more than 400 clinical samples using the Revised European/American Lymphoma (REAL) classification performed at more than thirty prominent independent testing centers throughout Europe in a collaborative study known as the BIOMED-2 Concerted Action. Results from this BIOMED-2 study indicate that testing for both *IGH* and *IGK* gene rearrangements may improve the reliability and sensitivity of the tests.[2] In addition, testing for both *IGH* and *IGK* gene rearrangements led to 99% sensitivity, compared to 88% for *IGH* and 88% for *IGK* when tested alone, which may also increase the reliability of the tests, improving the likelihood of detecting clonal products in more than one tube.[4]

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 six (6) master mixes. The *IGH* Tube A, B, and C master mixes target the framework 1, 2, and 3 regions (respectively) within the variable region, and the joining region of the immunoglobulin heavy chain locus. The *IGK* Tube A master mix targets the variable (V) and the joining (J) regions of the Ig kappa light chain locus. Whereas the *IGK* Tube B master mix targets kappa deleting element (K_{de}) rearrangements with the variable (V) region and the intragenic Jκ-Cκ region. The resulting Vκ-K_{de} and Jκ-Cκ intron-K_{de} rearrangements are a result of unsuccessful rearrangements retained by the B-cell. Lastly, 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 (bp) to ensure that the quality and quantity of input DNA is adequate to yield a valid result. A single thermocycler program and similar detection methodologies are used with all of our Gene Clonality Assays. This 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.

EuroClonality

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) and joining (J) regions (*IGH* Tubes A, B, and C), the variable (V) and joining (J) regions (*IGK* Tube A) and the variable, Jκ-Cκ intron, and K_{de} regions (*IGK* Tube B). 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 chain 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 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, and on a gel this distribution is seen as a smear. This Gaussian distribution reflects the heterogeneous population of V-J rearrangements. DNA from samples containing a clonal population yield one or two prominent amplified products (amplicons) within a diminished polyclonal background.

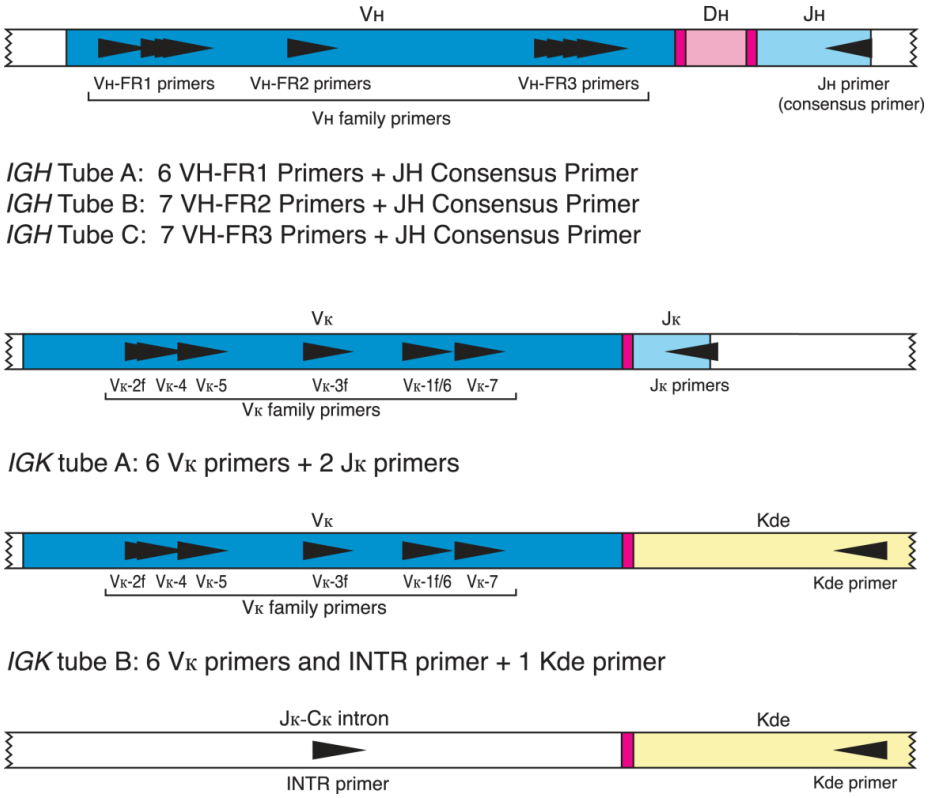

Figure 1. Depicted is a simple representation of the organization of a rearranged immunoglobulin heavy chain *(IGH)* gene on chromosome 14 and the immunoglobulin kappa light chain gene on chromosome 2p11.2. Black arrows represent the relative positions of primers that target the conserved framework (FR1-3) and the downstream consensus JH gene segments for *IGH* and the VK, JK, INTR and K_{de} primers which are included in the *IGK* master mix tubes.. The amplicon products generated from each of these regions can be differentially detected when fluorescent primer sets are used with capillary electrophoresis instruments that employ differential fluorescence detection.

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. Note that the primers that amplify the different FR regions, which are located at three distinct sections along the heavy chain gene, produce a correspondingly different size-range of V-J products. For rearrangements of the *IGK* gene, the length of the CDR3 region is limited and display significant skewing (platykurtosis). Thus, PCR products display a very narrow Gaussian distribution and are most easily and reliably identified by heteroduplex analysis.

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).

4. Reagents

4.1. Reagent Components

Table 1: Available Assays

Catalog Number	Description	Quantity
REF 91000010	IdentiClone *IGH* + *IGK* B-Cell Clonality Assay – Gel Detection	33 Reactions

Table 2. Kit Components

Reagent	Catalog #	Reagent Components (active ingredients)	Unit Quantity	# of Units	Storage Temp.
Master Mixes	21010010CE	***IGH* Tube A – Unlabeled** Multiple oligonucleotides targeting the framework 1 region of the immunoglobulin heavy chain gene in a buffered salt solution.	1500 µL	1	-85°C to -65°C
	21010020CE	***IGH* Tube B – Unlabeled** Multiple oligonucleotides targeting the framework 2 region of the immunoglobulin heavy chain gene in a buffered salt solution.	1500 µL	1	
	21010030CE	***IGH* Tube C – Unlabeled** Multiple oligonucleotides targeting the framework 3 region of the immunoglobulin heavy chain gene in a buffered salt solution.	1500 µL	1	
	21020010CE	***IGK* Tube A - Unlabeled** Multiple oligonucleotides targeting the variable and joining regions of the immunoglobulin kappa light chain gene in a buffered salt solution.	1500 µL	1	
	21020020CE	***IGK* Tube B - Unlabeled** Multiple oligonucleotides targeting the variable, Jκ-Cκ intron, and K_{de} regions of the immunoglobulin kappa light chain gene in a buffered salt solution.	1500 µL	1	
Template Amplification Control Master Mix	20960020	**Specimen Control Size Ladder – Unlabeled** Multiple oligonucleotides targeting housekeeping genes.	1500 µL	1	
	40881750	**IVS-0030 Clonal Control DNA** 200 µg/mL of DNA in 1/10th TE solution	100 µL	1	2°C to 8°C

Table 2. Kit Components

Reagent	Catalog #	Reagent Components (active ingredients)	Unit Quantity	# of Units	Storage Temp.
Positive Control DNAs	40881090	**IVS-0019 Clonal Control DNA** 200 µg/mL of DNA in 1/10th TE solution	100 µL	1	or -85°C -65°C
	40880370	**IVS-0007 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	

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. Use care to ensure use of correct thermocycler 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
- Suggested Instrument: Veriti™ thermal cycler or equivalent
- Performance characteristics and specification:
  - Minimum Thermal Range: 15°C to 96°C
  - Minimum Ramping Speed: 0.8°C/sec
- Follow manufacturer's installation, operation, calibration, and maintenance procedures.
- See section 7.4 *Amplification* for thermal cycler program.

5.2. 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** 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)
- 3 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 at 2°C to 8°C or at -85°C to -65°C until use.

7. Assay Procedure

7.1. Materials Provided

Table 3. Kit Components

Catalog #	Description
21010010CE	*IGH* Tube A – Unlabeled
21010020CE	*IGH* Tube B – Unlabeled
21010030CE	*IGH* Tube C – Unlabeled
21020010CE	*IGK* Tube A – Unlabeled
21020020CE	*IGK* Tube B – Unlabeled
20960020	Specimen Control Size Ladder – Unlabeled
40881750	IVS-0030 Clonal Control DNA
40881090	IVS-0019 Clonal Control DNA
40880370	IVS-0007 Clonal Control DNA
40920010	IVS-0000 Polyclonal Control DNA

7.2. Materials Required (not provided)

Table 4. Materials Required (not provided)

Reagent/Material	Recommended Reagents/Materials and Suppliers	Catalog #	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	Thermo Fisher Scientific: • Veriti Dx Thermal Cycler Bio-Rad: • 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	Invitrogen: • UltraPure™ 10 mg/mL Ethidium Bromide	15585-011	N/A
6% Polyacrylamide Gels	Invitrogen: • Novex® TBE Gels (6%, 12 well)	EC62652Box	N/A
TBE Running Buffer	Invitrogen: • NovexTBE Running Buffer (5X)	LC6675	Dilute 1:5 prior to use.

Table 4. Materials Required (not provided)

Reagent/Material	Recommended Reagents/Materials and Suppliers	Catalog #	Notes
Gel Loading Buffer	Invitrogen: • 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 (Cat#)	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

- All unknown samples can be tested using the Specimen Control Size Ladder master mix to 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, **duplicate** testing is reccommended 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 for each master mix.
- It is recommended to batch multiple samples in a run to avoid running out of the negative control (IVS-0000 Polyclonal Control DNA). If batching samples is not practical in your laboratory setting, IVS-0000 Polyclonal Control DNA is also available for purchase separately.

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 × 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 × 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 × 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 thermocycler.
- 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 thermocyclers.

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 thermocycler.

- 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 – 7.5.3 and proceed with step 7.5.4.

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* below)

7.6. Quality Control

Positive and negative (or normal) controls are furnished with the kit and are to 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 PCR 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 3100 platform.

Table 6. Expected Sizes of Recommended Positive Controls

Master Mix	Target	Control DNA	Catalog #	Product Size in Base pair (bp)
***IGH* Tube A**	FR1-J_H	**Valid Size Range** IVS-0030 Clonal Control DNA	--- 40881750	**310 - 360** 280[a], 325
***IGH* Tube B**	FR2-J_H	**Valid Size Range** IVS-0030 Clonal Control DNA	--- 40881750	**250 - 295** 260
***IGH* Tube C**	FR3-J_H	**Valid Size Range** IVS-0019 Clonal Control DNA	--- 40881090	**100 - 170** 145
***IGK* Tube A**	V_κ-J_κ	**Valid Size Range** IVS-0007 Clonal Control DNA	--- 40880370	**120 - 160, 190 - 210, 260 - 300** 143
***IGK* Tube B**	V_κ-K_{de} + intron-K_{de}	**Valid Size Range** IVS-0007 Clonal Control DNA	--- 40880370	**210 - 250, 270 - 300, 350 - 390** 274, 282
Specimen Control Size Ladder	Multiple Genes	**Valid Size Range** IVS-0000 Polyclonal Control DNA	--- 40920010	**100, 200, 300, 400, 600**[b] 100, 200, 300, 400, 600[b]

[a]**Note**: A 280 bp band may also be present and is a known amplicon that lies just outside the valid size range for *IGH* Tube A.
[b]**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 of the master mixes 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 of the master mixes.

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 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:

- One or two prominent positive bands[a] within the valid size range are reported as:
"**Positive for the detection of clonal immunoglobulin heavy chain or kappa 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 bands[a] within the valid size range is reported as:
"**Negative for the detection of clonal immunoglobulin heavy chain or kappa 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 3100 platform.

Table 8. Expected sies of amplified products

Master Mix	Target	Control DNA	Catalog #	Product Size in bp
***IGH* Tube A**	FR1-J_H	**Valid Size Range** IVS-0000 Polyclonal Control DNA IVS-0030 Clonal Control DNA IVS-0019 Clonal Control DNA IVS-0024 Clonal Control DNA IVS-0008 Clonal Control DNA	--- 40920010 40881750 40881090 40881390 40880430	**310 - 360** 310 - 360 280[a], 325 345 342 ---
***IGH* Tube B**	FR2-J_H	**Valid Size Range** IVS-0000 Polyclonal Control DNA IVS-0030 Clonal Control DNA IVS-0019 Clonal Control DNA IVS-0024 Clonal Control DNA IVS-0008 Clonal Control DNA	--- 40920010 40881750 40881090 40881390 40880430	**250 - 295** 250 - 295 260 285 277 ---
***IGH* Tube C**	FR3-J_H	**Valid Size Range** IVS-0000 Polyclonal Control DNA IVS-0030 Clonal Control DNA IVS-0019 Clonal Control DNA IVS-0024 Clonal Control DNA IVS-0008 Clonal Control DNA	--- 40920010 40881750 40881090 40881390 40880430	**100 - 170** 100 - 170 --- 145 --- ---
***IGK* Tube A**	V_κ-J_κ	**Valid Size Range** IVS-0000 Polyclonal Control DNA IVS-0007 Clonal Control DNA	--- 40920010 40880370	**120 - 160, 190 - 210, 260 - 300** 135 - 155 143
***IGK* Tube B**	V_κ-K_{de} + intron-K_{de}	**Valid Size Range** IVS-0000 Polyclonal Control DNA IVS-0007 Clonal Control DNA	--- 40920010 40880370	**210 - 250, 270 - 300, 350 - 390** 225-245, 265-285, 404[b] 274, 282
Specimen Control Size Ladder	Multiple Genes	**Valid Size Range** IVS-0000 Polyclonal Control DNA	--- 40920010	**100, 200, 300, 400, 600[c]** 100, 200, 300, 400, 600[c]

[a]**Note**:: A 280 bp band may also be present and is a known amplicon that lies just outside the valid size range for *IGH* Tube A.

[b]**Note**: Under sub-optimal conditions a nonspecific product of 404 bp can be detected in Tube B. To discriminate between specific and nonspecific, negative control DNA will not show this band within the same experiment. If a band is present, consider the band nonspecific.

[c]**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 mixes indicated. Amplified products were heteroduplexed and run on a 6% polyacrylamide gel.

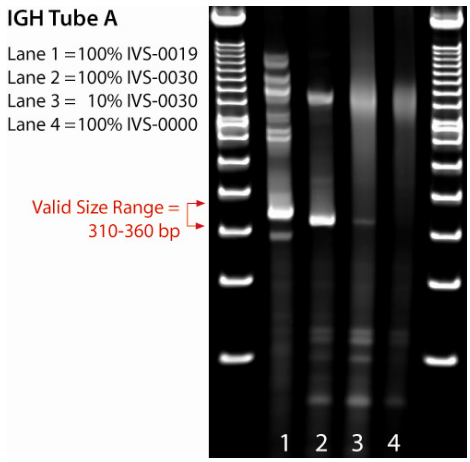

Figure 2. *IGH* Tube A

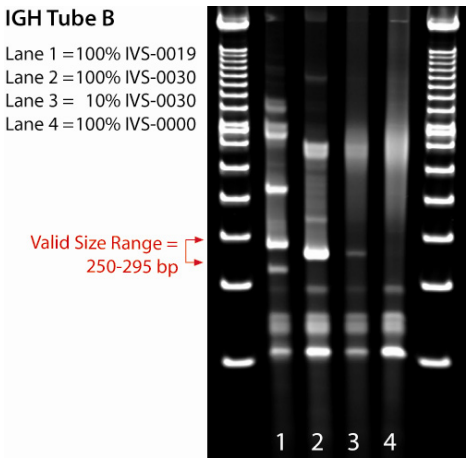

Figure 3 *IGH* Tube B

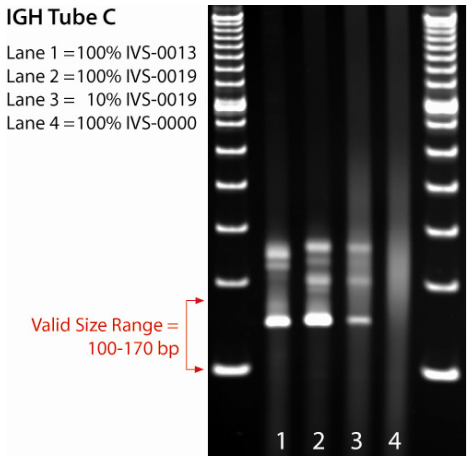

Figure 4 *IGH* Tube C

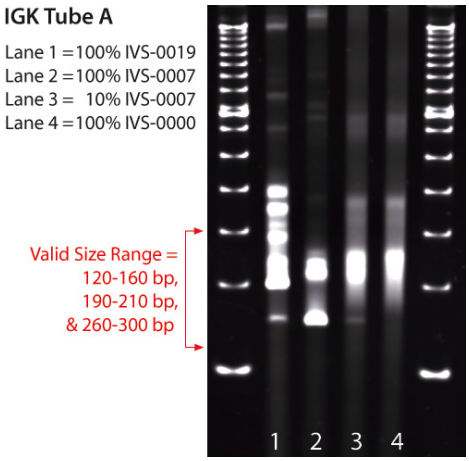

Figure 5 *IGK* Tube A

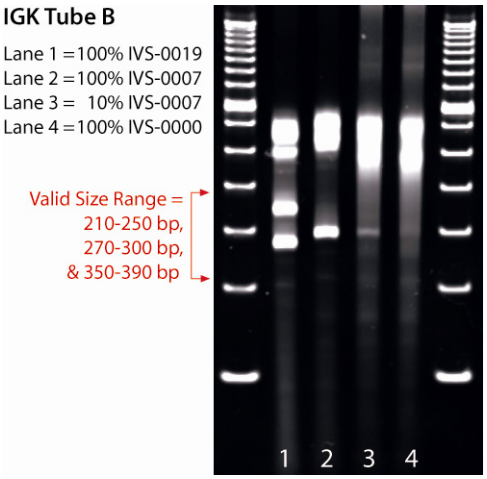

Figure 6. *IGK* Tube B

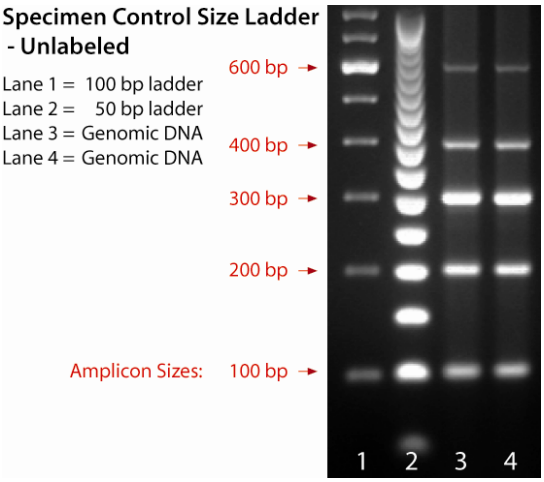

Figure 7. The data shown above were generated using the Specimen Control size Ladder master mix. Amplified products were run on a 2% agarose gel.

11. Performance Characteristics

This IdentiClone *IGH + IGK* B-Cell 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 evidenced by two notable papers, mentioned below.[2,3]

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%

An independent study by Sandberg *et al.* included 300 patient samples from a variety of sample types.[3] In cases where both PCR and SB analyses were done and results could be correlated with histopathology and a final diagnosis, the diagnostic accuracy of selected IdentiClone tests was determined to be at least 96%. This was far more accurate than SB analysis, which in this study missed 23 clear cases of malignancy, and seven (7) probable malignancies. There were no clear false positive results generated using the IdentiClone tests and there was a high level of precision.[3] 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. References

1. Miller, JE, Wilson, SS, Jaye, DJ, Kronenberg, M. (1999). An automated semiquantitative B and T cell clonality assay. *Molecular Diagnostics* 4, 101-117.
2. Van Dongen, JJM *et al.* (2003). 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*. 17, 2257-2317.
3. Sandberg, Y, *et al.* (2005). BIOMED-2 multiplex immunoglobulin/T-cell receptor polymerase chain reaction protocols can reliably replace Southern Blot analysis in routine clonality diagnostics. *J. Molecular Diagnostic* 7, 495-503.
4. van Krieken, JHJM *et al.* (2007). Improved reliability of lymphoma diagnostics via PCR-based clonality testing: – Report of the BIOMED-2 Concerted Action BHM4-CT98-3936. *Leukemia* 21, 201-206.

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		

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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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