Instructions for Use

BCL2/J_H Translocation Assay

RUO

PCR Assay for identification of *BCL2*/J_H translocations.

RUO This assay is for Research Use Only. Not for use in diagnostic procedures.

Manufactured in U.S.A.

invivoscribe

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

Catalog#	Products	Quantity
REF 13090020	*BCL2*/J_H Translocation Assay for Gel Detection	33 reactions
REF 13090040	*BCL2*/J_H Translocation Assay MegaKit for Gel Detection	330 reactions

Table of Contents

1. Assay Use

This Research Use Only assay identifies *BCL2*/J_H t(14;18) translocations.

2. Summary and Explanation of the Test

2.1. Background

BCL2 translocations are reciprocal chromosome exchanges that place the *bcl*-2 proto-oncogene, located on chromosome 18, under aberrant transcriptional control of the immunoglobulin heavy chain gene, located on chromosome 14. The *bcl*-2 protein is an antagonist to apoptosis (programmed cell death), a normal process designed to eliminate unneeded and damaged cells during hematopoiesis. Increased expression of the *bcl*-2 protein leads to an increase in the levels of B cells in the body.

2.2. Summary

This *BCL2* t(14;18) Translocation Assay contains four (4) master mixes. Primers in three (3) of the master mixes target the joining region of the immunoglobulin heavy chain gene and distinct regions of the *BCL2* gene. These master mixes are used to detect major breakpoint region (Mbr) and minor cluster region (mcr) of the *BCL2* t(14;18) translocations. The remaining master mix, the Specimen Control Size Ladder, targets multiple genes and generates a series of amplicons of 96, 197, 297, 397, and 602 base pairs (bp) to ensure DNA of sufficient quality and quantity was present to generate a valid result. Positive and negative control DNA are also included.

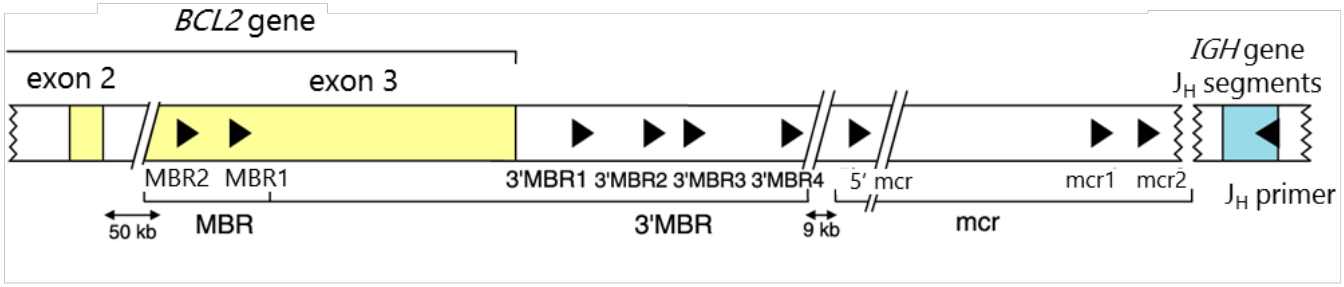
This assay is based on the EuroClonality/BIOMED-2 Concerted Action BMH4-CT98-3936.

EuroClonality

3. Principles of the Procedure

3.1. Polymerase Chain Reaction (PCR)

PCR assays are routinely used for the identification of chromosome translocations. This test targets the Mbr and mcr regions of the *BCL2*/J_H translocations (see Figure 1) and amplifies genomic DNA between primers that target the *BCL2* gene and the conserved joining (J) regions of the *IGH* gene (*BCL2*/J_H Tubes A, B, and C). Breakpoints that occur outside the Mbr and mcr regions will not be identified by this particular test. Therefore, a negative result does not completely exclude the presence of a *BCL2*/J_H gene rearrangement in the sample.[1] DNA from a normal lymphocyte population will also generate a negative result.



t(14;18) Tube A: two (2) *BCL2* MBR primers + one (1) J_H primer
t(14;18) Tube B: four (4) *BCL2* 3'MBR primers + one (1) J_H primer
t(14;18) Tube C: three (3) *BCL2* mcr primers + one (1) J_H primer

Figure 1. Depicted is a schematic diagram of the *BCL2*/J_H t(14;18) translocation showing the *BCL2* gene on the left and the Ig heavy chain (*IGH*) gene on the right. Shown are the relative positions and orientations for the major breakpoint region (Mbr) primers, the minor cluster region (mcr) primers, and the J_H primer, which are included in the 3 *BCL2*/J_H master mix tubes.

3.2. Gel Detection

Gel electrophoresis, such as agarose gel electrophoresis or non-denaturing polyacrylamide gel electrophoresis (PAGE), is commonly used to resolve amplicon products based on their size, charge, and conformation. Since DNA is negatively charged, when an electrical potential (voltage) is applied across the gel containing PCR products, the electrical field causes the amplicons to migrate through the gel. Smaller DNA fragments are able to easily migrate through the gel matrix, whereas larger DNA fragments migrate more slowly. This causes a separation of the amplicon products based on size. Ethidium bromide or other DNA intercalating dyes can then be used to stain and detect these products in the gel.

4. Reagents

4.1. Reagent Components

Table 1. Available Kits

Catalog #	Description	Total Reactions
REF 13090020	*BCL2*/J_H Translocation Assay for Gel Detection	33 Reactions
REF 13090040	*BCL2*/J_H Translocation Assay MegaKit for Gel Detection	330 Reactions

Table 2. Reagent Components

Reagent	Catalog #	Reagent Components (active ingredients)	Unit of Quantity	13090020 # of Units	13090040 # of Units	Storage Temp.
Master Mixes	23090050	***BCL2*/J_H Tube A – Unlabeled** Multiple oligonucleotides targeting the major breakpoint region (Mbr) of the *BCL2* gene and the J region of the *IGH* gene in a buffered salt solution.	1500 µL	1	10	-85°C to -65°C
	23090060	***BCL2*/J_H Tube B – Unlabeled** Multiple oligonucleotides targeting the 3' major breakpoint region (3' Mbr) of the *BCL2* gene and the J region of the *IGH* gene in a buffered salt solution.	1500 µL	1	10	
	23090070	***BCL2*/J_H Tube C – Unlabeled** Multiple oligonucleotides targeting the minor cluster region (mcr) of the *BCL2* gene and the J region of the *IGH* gene in a buffered salt solution.	1500 µL	1	10	
Template Amplification Control Master Mix	20960020	**Specimen Control Size Ladder – Unlabeled** Multiple oligonucleotides targeting housekeeping genes.	1500 µL	1	10	
Positive Controls	40881750	**IVS-0030 Clonal Control DNA** 200 µg/mL of DNA in 1/10th TE solution	100 µL	1	5	2°C to 8°C or -85°C to -65°C
	40900070	**IVS-P002 Clonal Control DNA** 1600 pg/mL of plasmid DNA diluted in IVS-0000 polyclonal control DNA in 1/10th TE solution	100 µL	1	5	
	40881810	**IVS-0031 Clonal Control DNA** 200 µg/mL of DNA in 1/10th TE solution	100 µL	1	5	
Negative (Normal) Control DNA	40920010	**IVS-0000 Polyclonal Control DNA** 200 µg/mL of DNA in 1/10th TE solution	100 µL	1	5	

4.2. Warnings and Precautions

- Use this assay kit as a system; do not substitute other manufacturers' reagents. Dilution, reducing amplification reactions or other deviations from this protocol may affect the performance of this test and/or nullify any limited sublicense that come with the purchase of these kits.
- Do not mix or combine reagents from kits with different lot numbers.
- Materials are stable until the labeled expiration date when stored and handled as directed. Do not use kits beyond their expiration date.
- Adherence to the protocol will assure optimal performance and reproducibility. Ensure correct thermal cycler programs are used, as other programs may provide inaccurate/faulty data such as false-positive and false-negative results.
- Dispose of unused reagents and waste in accordance with country, federal, state and local regulations.
- Perform all laboratory procedures with standard personal protective equipment (gloves, laboratory coats and protective eyewear). Follow good laboratory practices and universal precautions when working with specimens. Do not pipette by mouth. Do not eat, drink or smoke in laboratory work areas. Wash hands thoroughly after handling specimens and assay reagents.
- Handle specimens in approved biological safety containment facilities and open only in certified biological safety cabinets. Use molecular biology grade water for the preparation of specimen DNA.
- Due to the high analytical sensitivity of this test, use extreme care to avoid any contamination of reagents or amplification mixtures with samples, controls or amplified materials. Use fresh, aerosol-resistant pipette tips between samples and between dispensing reagents. Closely monitor all reagents for signs of contamination (*e.g.,* negative controls giving positive signals). Discard any reagents suspected of contamination.
- To minimize contamination, wear clean gloves when handling samples and reagents and routinely clean work areas and pipettes prior to setting up PCR.
- Follow uni-directional workflow between separate work areas in the PCR laboratory: begin with master mix preparation, move to specimen preparation, then to amplification and finally to detection. Autoclaving does not eliminate DNA contamination. Perform pre- and post-PCR steps in separate spaces. Avoid taking paper and other materials from post-PCR into the pre-PCR space.
- Dedicate all pipettes, pipette tips and any equipment used in a particular area to that area of the laboratory.
- Decontaminate non-disposable items with 10% bleach and rinse with distilled water two separate times before returning them to the starting areas.
- Use sterile, disposable plastic ware whenever possible to avoid contamination.

4.3. Storage and Handling

- Store the assay at **-85ºC to -65ºC** until ready to use.
- 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 completely resuspended.
- Due to high salt concentrations, PCR master mixes are sensitive to freeze/thaw cycles. Minimize the exposure of master mixes to freeze/thaw cycles.

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). Common sources of gDNA include

- 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)
- Formalin-fixed paraffin embedded tissue or slides (stored and shipped at ambient temperature)

6.4. Sample Preparation

Extract the gDNA from 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 was 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 3: Kit components

Catalog #	Description
REF 23090050	*BCL2*/J_H Tube A – Unlabeled
REF 23090060	*BCL2*/J_H Tube B – Unlabeled
REF 23090070	*BCL2*/J_H Tube C – Unlabeled
REF 20960020	Specimen Control Size Ladder – Unlabeled
REF 40881750	IVS-0030 Clonal Control DNA
REF 40900070	IVS-P002 Clonal Control DNA
REF 40881810	IVS-0031 Clonal Control DNA
REF 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 # (REF)	Notes
DNA Polymerase	Roche: • EagleTaq DNA Polymerase Invivoscribe: • 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	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

Table 4: Materials Required (not provided)

Reagent/Material	Recommended Reagents/Materials and Suppliers	Catalog # (REF)	Notes
100 bp DNA Ladder	Thermo Fisher Scientific: • TrackIt™ 100 bp DNA Ladder	10488-058	N/A

7.3. Reagent Preparation

- Test all unknown samples with 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.
- Test all samples in **singlicate**.
- 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; then gently vortex to mix.

7.3.2. In containment hood or dead air box remove an appropriate aliquot to clean, sterile microfuge tube (one tube for each master mix).

- Aliquot volumes are 45 µL for each sample + 135 µL (3 x 45 µL) for the positive, negative and no template controls.
- Include an additional 20 µL to correct for pipetting errors.

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

- The master mixes are now ready for distribution to reaction tubes or plate and addition of sample.

7.4. Amplification

7.4.1. Aliquot 45 µL of the master mix/enzyme solutions into individual PCR wells or tubes.

7.4.2. Add 5 µL sample or control DNA to the individual tubes or wells containing the respective master mix reactions.

- Pipette up and down several times to mix.

7.4.3. Amplify the reactions using the following PCR program:

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

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

7.4.4. Remove the amplification plate from the thermal cycler

7.5. Detection

Gel Detection – Agarose TBE Gels (RECOMMENDED)

7.5.1. Prepare a 2% MetaPhor or NuSieve 3:1 agarose/TBE gel.

7.5.2. Individually mix 20 µL from each amplification reaction with 4 µL of 6X gel loading buffer.

7.5.3. Load 20 µL of this mixture into separate wells of the gel, flanked by DNA size standards.

7.5.4. Detect products using ethidium bromide or an equivalent dye.

7.5.5. Photograph gel and interpret data.

Gel Detection – Polyacrylamide TBE Gels

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

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

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

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

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

7.5.10. Gels are stained in 0.5 µg/mL ethidium bromide (in water or 0.5X TBE Buffer) for 5-10 minutes.

7.5.11. De-stain gels 2X in water for 5-10 minutes.

7.5.12. Visualize gels using UV illumination.

7.5.13. Photograph gel and interpret data.

Gel Detection – Heteroduplex Analysis

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

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

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

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

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

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

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

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

7.5.21. De-stain gels 2X in water for 5-10 minutes.

7.5.22. Visualize gels using UV illumination.

7.5.23. Photograph gel and interpret data.

7.6. Available Template Amplification Controls

The **Specimen Control Size Ladder** master mix generates amplicons at 96, 197, 297, 397 and 602 bp.

- The ~100 bp band is comprised of 84 bp and 96 bp bands. Both of these bands co-migrate on a gel.
- Run the products of this master mix separately.

7.7. Recommended Positive Controls

Table 6: Recommended Positive Controls

Master Mix	Target	Control DNA	Catalog #	Product Size in base pairs (bp)
***BCL2*/J_H Tube A**	Mbr of *BCL2*/J_H	**Valid Size Range** IVS-0030 Clonal Control DNA	--- 40881750	**100 - 2500** ~250, ~750[a]
***BCL2*/J_H Tube B**	3' Mbr of *BCL2*/J_H	**Valid Size Range** IVS-P002 Clonal Control DNA	--- 40900070	**100 - 2500** 139
***BCL2*/J_H Tube C**	mcr of *BCL2*/J_H	**Valid Size Range** IVS-0031 Clonal Control DNA	--- 40881810	**100 - 2500** ~390, ~800
Specimen Control Size Ladder	Multiple Genes	**Valid Size Range** IVS-0000 Polyclonal Control DNA	--- 40920010	**84, 96, 197, 297, 397, 602** 84, 96, 197, 297, 397, 602

[a]**Note:** The ~750 bp band (*BCL2*/J_H Tube A) and the ~800 bp band (*BCL2*/J_H Tube C) are generally weak bands.

8. Interpretation and Reporting

This assay is for research use only; not intended for diagnostic purposes. PCR based testing does not identify 100% of clonal cell populations; therefore, repeat testing by NGS may be advisable to rule out clonality.

8.1. Sample Interpretation

8.1.1. Samples that fail to amplify following repeat testing should be reported as "**A result cannot be reported on this specimen because there was DNA of insufficient quantity or quality for analysis**".

8.1.2. All assay controls must be examined prior to interpretation of sample results. If the controls do not yield the correct results, the assay is not valid and the samples cannot be interpreted.

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

- **Negative Control**: (Polyclonal control, water or no template blank). If the negative control is:
  - **Positive**: Possible contamination of all PCR amplification reactions. Do not continue with the interpretation of results. Prepare fresh master mix and repeat amplification.
  - **Negative**: Continue with the analysis.
- **Positive Control**: (This can also be an extraction control if positive control material is taken through extraction processes). If the positive control is:
  - **Positive:** Continue with analysis.
  - **Negative:** Repeat the assay.
- **Specimen Control Size Ladder**: (This is run on unknown samples only). If the amplification control:
  - **Generates 96, 197, 297, 397 and 602 bp products:** Because smaller PCR fragments are preferentially amplified, it is not unusual for the 602 bp fragment to have a diminished signal or to be missing entirely. Continue with analysis.
  - **Does not generate indicated products**: Repeat the assay and/or re-extract the specimen.

8.2. Sample Reporting

Following the acceptance of the controls, the samples are interpreted as follows: One or two prominent bands within the 100 - 2500 bp range are reported as "**Detection of a *BCL2*/J_H t(14;18) Translocation**".

9. Limitations of Procedure

The assay is subject to interference by degradation of DNA or inhibition of PCR due to heparin or other agents.

10. Expected Results

10.1. Expected Size of Amplified Products

The size range for each master mix has been determined testing positive control samples. For accurate and meaningful interpretation, ignore bands that occur outside of the valid size range for each master mix. Bands outside of the range cannot be assumed to be valid.

Note: These master mixes use a consensus J_H primer. Therefore, a positive specimen can generate several valid products. These amplicons differ in size by about 500 bp which is the distance between intron segments between these tandem J_H exons.

Table 7: Expected Size of amplified Products

Master Mix	Target	Control DNA	Catalog #	Product Size in base pairs (bp)
***BCL2*/J_H Tube A**	Mbr of *BCL2*/J_H	**Valid Size Range** IVS-0000 Polyclonal Control DNA IVS-0030 Clonal Control DNA IVS-0031 Clonal Control DNA	--- 40920010 40881750 40881810	**100 - 2500** --- ~250, ~750[a] ---
***BCL2*/J_H Tube B**	3' Mbr of *BCL2*/J_H	**Valid Size Range** IVS-0000 Polyclonal Control DNA IVS-P002 Clonal Control DNA IVS-0030 Clonal Control DNA IVS-0031 Clonal Control DNA	--- 40920010 40900070 40881750 40881810	**100 - 2500** --- 138 --- ---
***BCL2*/J_H Tube C**	mcr of *BCL2*/J_H	**Valid Size Range** IVS-0000 Polyclonal Control DNA IVS-0030 Clonal Control DNA IVS-0031 Clonal Control DNA	--- 40920010 40881750 40881810	**100 – 2500** --- --- ~390, ~800
Specimen Control Size Ladder	Multiple Genes	**Valid Size Range** Any Human DNA	---	**84, 96, 197, 297, 397, 602** 84, 96, 197, 297, 397, 602

[a]**Note:** The ~750 bp band (*BCL2*/J_H Tube A) and the ~800 bp band (*BCL2*/J_H Tube C) are generally weak bands.y.

10.2. Sample Data

The data shown below were generated using the master mix indicated. Amplified products were run on a 2% agarose gel.

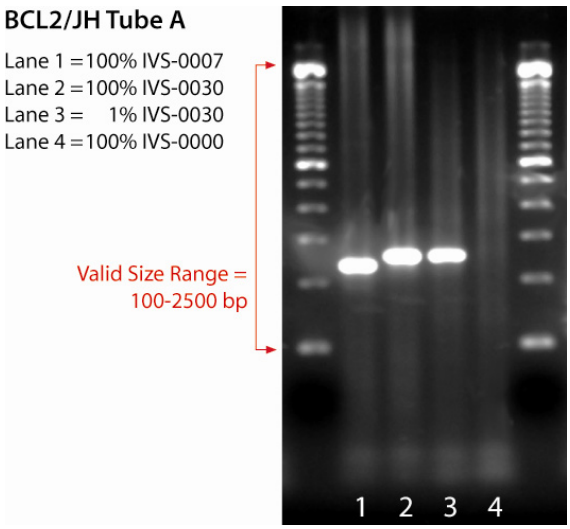


Figure 2. *BCL2*/J_H Tube A master mix.

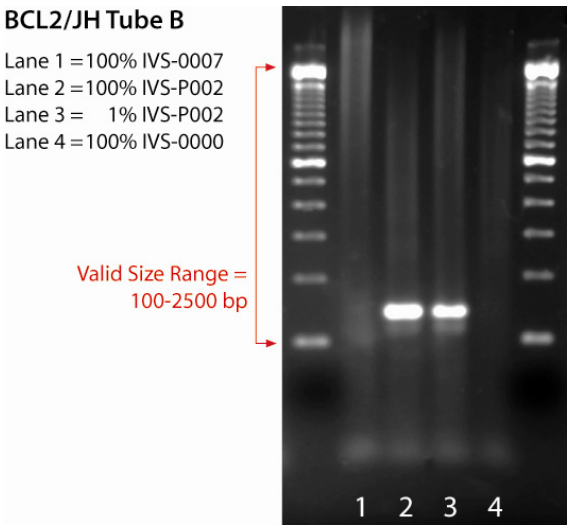


Figure 3. *BCL2*/J_H Tube B master mix.

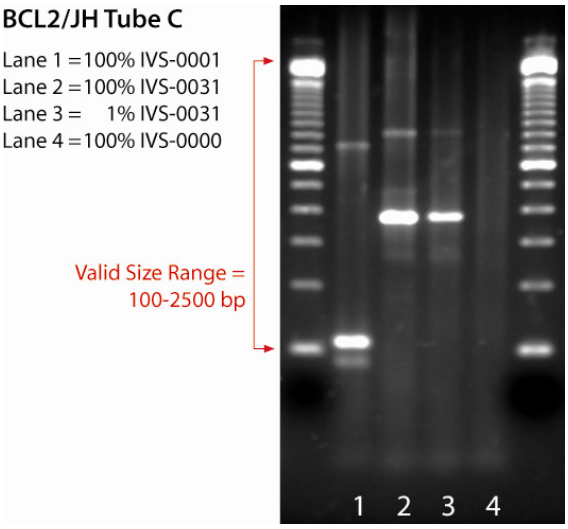


Figure 4. *BCL2*/J_H Tube C master mix.

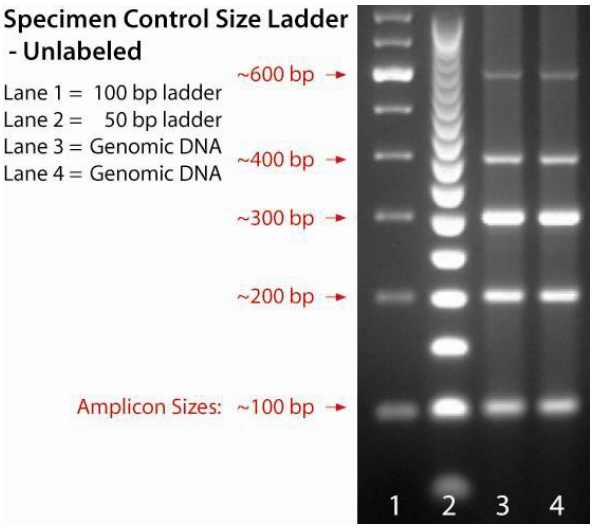


Figure 5. Specimen Control Size Ladder master mix.

11. Technical and Customer Service

Thank you for purchasing our ***BCL2*/J_H Translocation Assay**. We appreciate your business. We are happy to assist you in the validation of this assay and will provide ongoing technical assistance to keep the assays performing efficiently in your laboratory. Technical assistance is most rapidly obtained using our Internet site: **http://www.invivoscribe.com** or by sending an email inquiry to: **support@invivoscribe.com**.

Contact Information

Invivoscribe, Inc

10222 Barnes Canyon Road | Building 1 | San Diego | California 92121-2711 | USA

Phone: +1 858 224-6600 | Fax: +1 858 224-6601 | Business Hours: 7:00AM - 5:00 PM PST/PDT

Technical Service: support@invivoscribe.com | Customer Service: sales@invivoscribe.com | Website: www.invivoscribe.com

12. References

1. 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. Symbols

The following symbols are used in Invivoscribe product labeling.

REF	Catalog Number		Expiration Date
VOL	Reagent Volume		Manufacturer
LOT	Lot Number		Consult Instructions for Use
	Storage Conditions	RUO	Research Use Only

14. Legal Notice

14.1. Warranty and Liability

Invivoscribe, Inc. (Invivoscribe®) is committed to providing the highest quality products. Invivoscribe® warrants that the products meet or exceed the performance standards described in the Instructions For Use, as to products with such an insert. If a product is covered by product specifications and does not perform as specified, our policy is to replace the product or credit the full purchase price. No other warranties of any kind, expressed or implied, are provided by Invivoscribe®. Invivoscribe® liability shall not exceed the purchase price of the product. Invivoscribe shall have no liability for direct, indirect, consequential or incidental damages arising from the use, results of use, or inability to use its products; product efficacy under purchaser controlled conditions in purchaser's laboratory must be established and continually monitored through purchaser defined and controlled processes including but not limited to testing of positive, negative, and blank controls every time a sample is tested. Ordering, acceptance, and use of product constitutes purchaser acceptance of sole responsibility for assuring product efficacy and purchaser agreement to the limitation of liability set forth in this paragraph.

This product is for Research Use Only; not for use in diagnostic procedures.

14.2. Patents and Trademarks

This product is covered by one or more of the following: European Patent Number 1549764, European Patent Number 2418287, European Patent Number 2460889, Japanese Patent Number 4708029, United States Patent 8859748, and related pending and future applications. All of these patents and applications are licensed exclusively to Invivoscribe®. Additional patents licensed to Invivoscribe covering some of these products apply elsewhere. Many of these products require nucleic acid amplification methods such as Polymerase Chain Reaction (PCR). No license under these patents to use amplification processes or enzymes is conveyed expressly or by implication to the purchaser by the purchase of this product.

15. *BCL2*/J_H Translocation Assay: Single Page Guide

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

15.2. In a containment hood or dead air box remove an appropriate aliquot to clean, sterile microfuge tube (one tube for each of the master mixes).

- Aliquot volumes are 45 µL for each sample + 135 µL for the positive, negative and no template controls.
- Include an additional 20 µL to correct for pipetting errors.

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

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

15.5. Add 5 µL of DNA from the unknown and control samples to individual tubes or wells containing the respective master mix reactions and pipette up and down several times to mix.

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

Gel Detection – Agarose TBE Gels (RECOMMENDED)

15.7. Prepare a 2% MetaPhor or NuSieve 3:1 agarose/TBE gel.

15.8. Individually mix 20 µL from each amplification reaction with 4 µL of 6X gel loading buffer.

15.9. Load 20 µL of this mixture into separate wells of the gel, flanked by DNA size standards.

15.10. Detect products using ethidium bromide or an equivalent dye.

15.11. Photograph gel and interpret data.

Gel Detection – Polyacrylamide TBE Gels

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

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

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

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

15.16. Gels are stained in 0.5 µg/mL ethidium bromide (in water or 0.5X TBE Buffer) for 5-10 minutes.

15.17. De-stain gels 2X in water for 5-10 minutes.

15.18. Visualize gels using UV illumination.

15.19. Photograph gel and interpret data.

Gel Detection – Heteroduplex Analysis

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

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

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

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

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

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

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

15.27. De-stain gels 2X in water for 5-10 minutes.

15.28. Visualize gels using UV illumination.

15.29. Photograph gel and interpret data.