Instructions for Use

BCL2/J_H t(14;18) 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: **-85ºC to -65ºC**

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

Catalog#	Products	Quantity
REF 13090010	*BCL2*/J_H t(14;18) Translocation Assay for Gel Detection	30 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. Summary

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. Background

This *BCL2* t(14;18) Translocation Assay contains five (5) master mixes. Primers in four 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 *BCL2* t(14;18) translocations. The remaining master mix targets a ubiquitous HLA class II gene to ensure DNA of sufficient quality and quantity was present to generate a valid result. Positive and negative control DNAs are also included.

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.

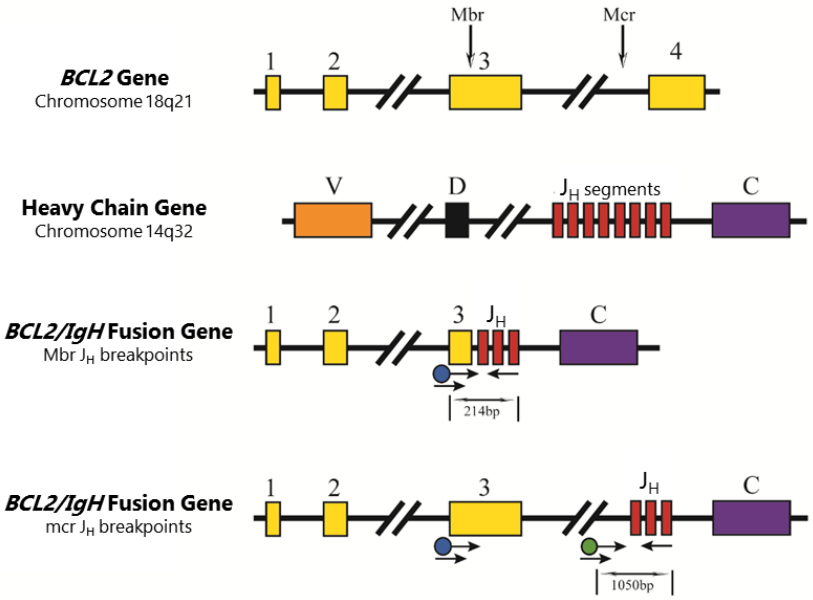

Figure 1. Simplified view of the genomic organization of the *BCL2* and *IGH* genes on chromosomes 18 and 14, respectively. Yellow boxes represent the exon regions of the *BCL2* gene. Exons of the immunoglobulin heavy chain gene are represented in other colors. The solid black lines represents intron regions, which have been left incompletely spliced to assist in demarcation of the exon segments. Mbr and mcr type t(14;18) translocations are shown in the lower portions of the figure with the relative positions of primers and the size of the amplicons generated from the positive control DNA indicated.

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 13090010	*BCL2*/J_H t(14;18) Translocation Assay for Gel Detection	30 Reactions

Table 2. Reagent Components for Gel Detection

Reagent	Catalog #	Reagent Components (active ingredients)	Unit of Quantity	# of Units	Storage Temp.
Master Mixes	23090030	***BCL2*/J_H t(14;18) (Mbr) Mix 1a – Unlabeled** 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	-85°C to -65°C
	23090010	***BCL2*/J_H t(14;18) (Mbr) Mix 1b – Unlabeled** 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	
	23090040	***BCL2*/J_H t(14;18) (mcr) Mix 2a – Unlabeled** Oligonucleotides targeting the 3' minor cluster region (mcr) of the *BCL2* gene and the J region of the *IGH* gene in a buffered salt solution.	1500 µL	1	
	23090020	***BCL2*/J_H t(14;18) (mcr) Mix 2b – Unlabeled** 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	
Template Amplification Control Master Mix	20960010	**Amplification Control – Unlabeled** Oligonucleotides targeting the HLA-DQα locus; the Major Histocompatibility Complex or Human Leukocyte Antigen (HLA), class II, DQ alpha 1.	1500 µL	1	
Controls and Standard	40881750	**IVS-0030 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
	40881810	**IVS-0031 Clonal Control DNA** 200 µg/mL of DNA in 1/10th TE solution	100 µL	1	
	40880490	**IVS-0009 Clonal Control DNA** 200 µg/mL of DNA in 1/10th TE solution	100 µL	1	

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.
- 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.

If you have any questions, please contact the Invivoscribe technical staff. We would be happy to help determine optimal storage needs.

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 35 V to 135 V for extended times
- Follow manufacturer's installation, operation, calibration, and maintenance procedures.

5.3. UV Illumination Unit (for Gel Detection)

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

6. Specimen Collection and Preparation

6.1. Precautions

Biological specimens from humans may contain potentially infectious materials. Handle all specimens in accordance with the Bloodborne Pathogen program and/or Biosafety Level 2 standards.

6.2. Interfering Substances

The following substances are known to interfere with PCR:

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

6.3. Specimen Requirements and Handling

This assay tests extracted and purified genomic DNA (gDNA). Common sources of gDNA include:

- 5 cc of peripheral blood, bone marrow biopsy or bone marrow aspirate anti-coagulated with heparin or EDTA; OR
- Formalin-fixed paraffin embedded tissue or slides.

6.4. Sample Preparation

This is a robust assay system. A wide range of DNA concentrations will generate a valid result. Quantifying and adjusting DNA concentrations is generally not necessary.

6.4.1. Using any method of DNA extraction, extract the gDNA from unknown samples.

6.4.2. Resuspend DNA to final concentration of 100 µg – 400 µg per mL in TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) or distilled water.

- When testing unknown samples, a template amplification control master mix (*e.g.,* Amplification Control or Specimen Control Size ladder) can be used to verify the absence of PCR inhibitors and sufficient quality and quantity of DNA is present to generate a valid result.

6.5. Sample Storage

Store samples using a method that prevents degradation of DNA.

7. Assay Procedure

7.1. Materials Provided

Please see Table 2 for a list of provided materials.

7.2. Materials Required (not provided)

Table 3. Materials Required (not provided)

Reagent/Material	Recommended Reagents/Materials and Suppliers	Part Number	Notes
DNA Polymerase	Roche: • EagleTaq DNA Polymerase Invivoscribe, Inc. • FalconTaq DNA Polymerase or equivalent	05206944190 60970130	N/A
Molecular Biology Grade or USP Water	N/A	N/A	DNase / RNase free
Calibrated Pipettes	N/A	N/A	Capable of accurately measuring volumes between 0.5 µL and 1000 µL.
Thermal cycler	Thermo Fisher Scientific: • Veriti Dx Thermal Cycler Bio-Rad: • MJ Research PTC-100, or PTC-200, PTC-220, or PTC-240 Perkin-Elmer • PE 2600, 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
Ethidium Bromide	Thermo Fisher Scientific: • UltraPure™ 10 mg/mL Ethidium Bromide	15585-011	N/A
Agarose Gels	Thermo Fisher Scientific: • MetaPhor™ Agarose, 125 g or • Lonza™ NuSieve™ 3:1 Agarose	BMA50180 or BMA50090	N/A
6% Polyacrylamide Gels	Thermo Fisher Scientific: • Novex® TBE Gels (6%, 12 well)	EC62652Box	N/A
TBE Running Buffer	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
Gel Electrophoresis Unit	N/A	N/A	For agarose or polyacrylamide gels

7.3. Reagent Preparation

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

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

- Aliquot volumes are 50 µL for each sample + 150 µL (3 x 50 µL) for the positive, negative, and no template controls.
- Add an additional 20 µL to correct for pipetting errors.

7.3.3. Add the appropriate amount of Taq DNA polymerase (0.25 µL @5 U/µL per 55 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.

7.3.4. Continue to step 7.4.1 for the single amplification protocol or step 7.4.5 for the nested amplification protocol.

7.4. Amplification

Single Amplification Protocol

- **Strategy**: Employ two Master Mixes to independently identify *BCL2* t(14;18) translocations involving the major breakpoint and minor cluster regions (Mbr and mcr, respectively).
- **Advantage**: Two separate Master Mixes are used to identify Mbr and mcr *BCL2* translocations.

7.4.1. Aliquot 50 µL of the master mix/enzyme solutions into individual PCR wells or tubes.

- *BCL2*/J_H t(14;18) Mbr Mix 1b and
- *BCL2*/J_H t(14;18) mcr Mix 2b

7.4.2. Add 5 µL of sample or control DNA to the individual tubes or wells containing the respective master mix reactions. Pipette up and down several times to mix.

7.4.3. Amplify the reactions using the PCR program in Table 4:

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

Table 4. Thermal cycling conditions

Standard Program for EagleTaq			
Step	**Temperature**	**Duration**	**Cycles**
1	95°C	7 minutes	1
2	94°C	30 seconds	35
3	55°C	30 seconds	
4	72°C	1 minute	
5	72°C	10 minutes	1
6	4°C	∞	1

7.4.4. Remove the amplification plate from the thermal cycler.

Nested Amplification Protocol

- **Strategy**: Employ an outer set of primers to amplify Mbr and mcr of *BCL2* t(14;18) translocation targets. Followed by secondary amplification with an internal primer set to detect final product.
- **Advantage**: Better sensitivity if translocation is identified.

7.4.5. Aliquot 50 µL of the 1st round master mix/enzyme solutions into individual PCR wells or tubes.

- *BCL2*/J_H t(14;18) Mbr Mix 1a and
- *BCL2*/J_H t(14;18) mcr Mix 2a

7.4.6. Add 5 □L of sample or control DNA to the individual tubes or wells containing the respective master mix reactions. Pipette up and down several times to mix.

7.4.7. Amplify the reactions using the appropriate PCR program in Table 4.

7.4.8. Remove the amplification plate from the thermal cycler.

7.4.9. Dilute the products from the first round of amplification 1:100 (2 µL + 198 µL of molecular biology grade distilled water).

- Mix thoroughly prior to adding to the prepared 2nd round amplification mixes

7.4.10. Repeat steps 7.4.1 - 7.4.4 for the 2nd round of amplification master mixes.

- *BCL2*/J_H t(14;18) Mbr Mix 1b and
- *BCL2*/J_H t(14;18) mcr Mix 2b

Note: For sample reactions, add the diluted amplification products to the following master mixes:

- *BCL2*/J_H t(14;18) Mbr Mix 1a product added into *BCL2*/J_H t(14;18) Mbr Mix 1b
- *BCL2*/J_H t(14;18) mcr Mix 2a product added into *BCL2*/J_H t(14;18) mcr Mix 2b

7.5. Detection

Available Template Amplification Controls

The **Amplification Control** master mix primers are available unlabeled for gel detection and labeled with a fluorescent dye (6-FAM) for ABI fluorescence detection. The 6-FAM label is detected as **BLUE** using the differential fluorescence software. The amplicons produced with this master mix are at 235 base pair (bp).

- Run the products of this master mix separately.

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 the gel and interpret the data.

Gel Detection – Polyacrylamide TBE Gels

7.5.6. Assemble electrophoresis unit using a 6% non-denaturing polyacrylamide TBE gel (made with 1X TBE) and 0.5X TBE running buffer.

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

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

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

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

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

7.6. Recommended Positive Controls

Table 5. Recommended positive controls and size of amplified products

Master Mix	Target	Control DNA	Catalog #	Product Size (bp)
***BCL2*/J_H t(14;18) Mbr Mix 1a**	Outside *BCL2* Mbr primers	**Valid Size Range** IVS-0030 Clonal Control DNA	--- 40881750	**550-800** ~800
***BCL2*/J_H t(14;18) Mbr Mix 1b**	Inside *BCL2* Mbr primers	**Valid Size Range** IVS-0030 Clonal Control DNA	--- 40881750	**80-300** 215
***BCL2*/J_H t(14;18) mcr Mix 2a**	Outside *BCL2* mcr primers	**Valid Size Range** IVS-0031 Clonal Control DNA	--- 40881810	**600-1200** ~1050
***BCL2*/J_H t(14;18) mcr Mix 2b**	Inside *BCL2* mcr primers	**Valid Size Range** IVS-0031 Clonal Control DNA	--- 40881810	**500-1100** ~850
Amplification Control	HLA-DQα	**Valid Size Range** IVS-0009 Clonal Control DNA	--- 40880490	**235** 235

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 Analysis

More than 85% of all clonal B cell populations are identified with this assay. However, due to the diversity of the antigen receptor genes and heterogeneity in translocation breakpoints, subsequent testing of negative samples by NGS may be advisable to rule out clonality.

Results can be reported as "Positive" or "Negative" for "Detection of *BCL2* t(14;18) translocation"

8.1.1. Samples that fail to amplify following repeat testing can 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.

8.1.3. The following describes the analysis of each control 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.

Note: There are several background peaks observed when testing the Polyclonal Negative Control and many normal samples, see detailed description, above.

  - **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 assay.
- **Amplification Control**: (This is run on unknown samples only). If the amplification control generates:
  - **235 bp products**: Continue with analysis.
  - **No peaks**: Repeat assay and/or obtain a new specimen.

8.2. Sample Interpretation

Following the acceptance of the controls, the samples are interpreted as follows:

8.2.1. *BCL2*/J_H t(14;18) (Mbr) Mix 1a:

Presence of a prominent band within the 550 - 800 bp range is reported as: "Detection of a *BCL2* t(14;18) Translocation involving the major breakpoint region of *BCL2*."

8.2.2. *BCL2*/J_H t(14;18) (Mbr) Mix 1b:

Presence of a prominent band within the 80 - 300 bp range is reported as: "Detection of a *BCL2* t(14;18) Translocation involving the major breakpoint region of *BCL2*."

8.2.3. *BCL2*/J_H t(14;18) (mcr) Mix 2a:

Presence of a prominent band within the 600 - 1200 bp range is reported as: "Detection of a *BCL2* t(14;18) Translocation involving the minor cluster region of *BCL2*."

8.2.4. *BCL2*/J_H t(14;18) (mcr) Mix 2b:

Presence of a prominent band within the 500 - 1100 bp range is reported as: "Detection of a *BCL2* t(14;18) Translocation involving the minor cluster region of *BCL2*."

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 Polyclonal Negative Control DNAs. For accurate and meaningful interpretation, it is important to ignore products that occur outside of the valid size range. Products that occur outside of the expected size range are not indicative of *BCL2* translocations.

Note: Products may display slightly different mobility in different gels and buffers. Therefore it is important to validate with the system that you intend to use for routine analysis.

Table 6. Expected Size of Amplified Products

Master Mix	Target	Control DNA	Cat#	Product Size (bp)
***BCL2*/J_H t(14;18) Mbr Mix 1a**	Outside *BCL2* Mbr primers	**Valid Size Range** IVS-0009 Clonal Control DNA IVS-0030 Clonal Control DNA	--- 40880490 40881750	**550-800** None[1] ~800
***BCL2*/J_H t(14;18) Mbr Mix 1b**	Inside *BCL2* Mbr primers	**Valid Size Range** IVS-0009 Clonal Control DNA IVS-0030 Clonal Control DNA	--- 40880490 40881750	**80-300** None[2] 215
***BCL2*/J_H t(14;18) mcr Mix 2a**	Outside *BCL2* mcr primers	**Valid Size Range** IVS-0009 Clonal Control DNA IVS-0031 Clonal Control DNA	--- 40880490 40881810	**600-1200** None[3] ~1050
***BCL2*/J_H t(14;18) mcr Mix 2b**	Inside *BCL2* mcr primers	**Valid Size Range** IVS-0009 Clonal Control DNA IVS-0031 Clonal Control DNA	--- 40880490 40881810	**500-1100** None[4] ~850
Amplification Control	HLA-DQα	Any Human DNA	---	235

Note: The amplicon sizes listed above were determined using an ABI platform.
[1]**Note:** Products of 150 bp and 500 bp may be observed testing the negative control and normal samples.
[2]**Note:** Products of 116 bp and 190 bp may be observed testing the negative control and normal samples. In nested applications amplicons at 525 bp and 625 bp may be observed.
[3]**Note:** Product of ~600 bp may be observed testing the negative control and normal samples.
[4]**Note:** Product of ~325 bp may be observed testing the negative control and normal samples. In nested applications amplicons at 525 bp and 625 bp may be observed.

10.2. Sample Data

Gel Detection: The data shown in Figure 2 - Figure 6 were generated using the master mixes indicated.

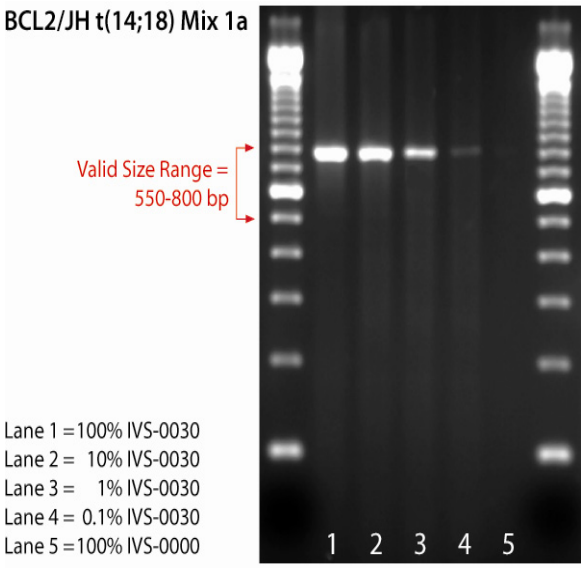

Figure 2. Amplified products were generated with the *BCL2*/J_H t(14;18) Master Mix 1a then run on a 2% agarose gel.

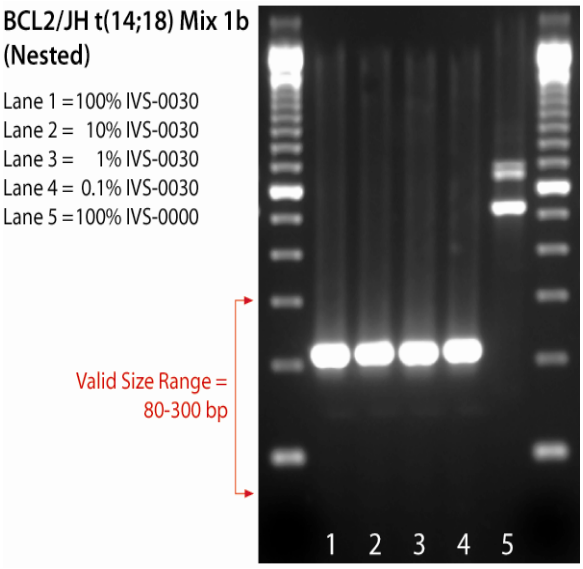

Figure 3. Amplified products were generated with the *BCL2*/J_H t(14;18) Master Mix 1b then run on a 2% agarose gel.

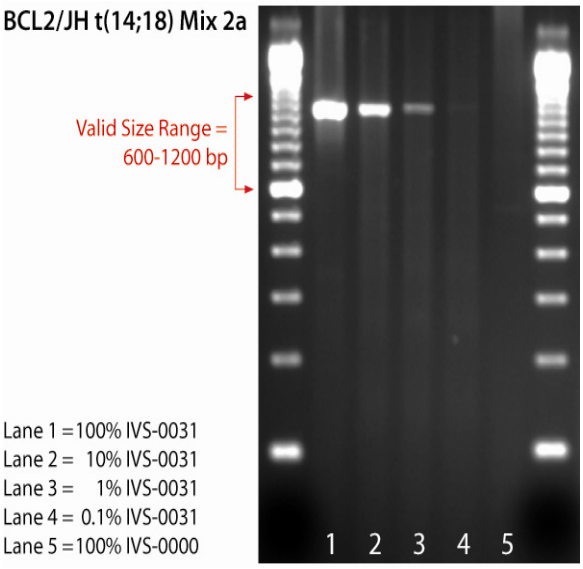

Figure 4. Amplified products were generated with the *BCL2*/J_H t(14;18) Master Mix 2a then run on a 2% agarose gel.

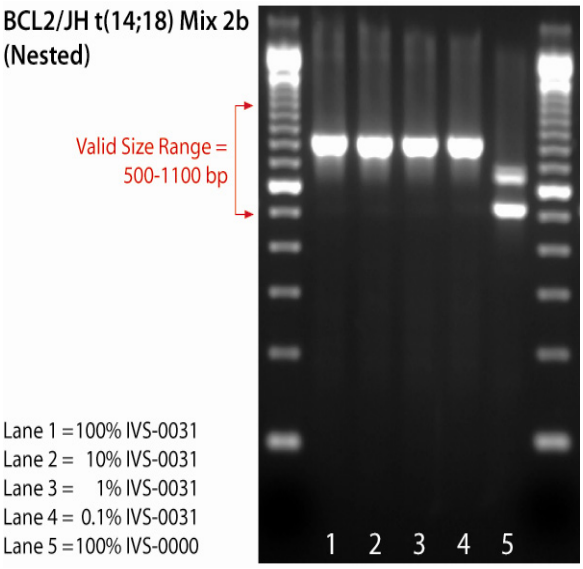

Figure 5. Amplified products were generated with the *BCL2*/J_H t(14;18) Master Mix 2b then run on a 2% agarose gel.

These images were generated with IVS-0000 Polyclonal Control DNA; however, IVS-0009 clonal control DNA is also negative for *BCL2*/J_H t(14;18) translocations and will yield similar results when used as the negative control.

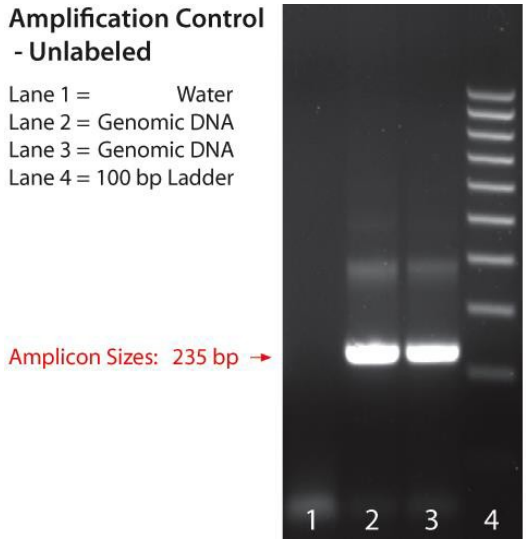

Figure 6. This is a photograph of a 2% agarose/TAE gel with data generated from two different genomic DNA samples (lanes 2 and 3). Both samples generate a bright band at 235 bp.

11. Technical and Customer Service

Thank you for purchasing our ***BCL2*/J_H t(14;18) 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

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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. Deininger, MWN, Goldman, JM and JV Melo. *Blood*, 2000, **96**:3343-3356.
2. Miller, JE (Application filed: US Patent and Trademark Office)
3. Miller, JE, Wilson, SS, Jaye, DL and Kronenberg, M. *Molecular Diagnosis*, 1999, **4**: 101-117.

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

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15. *BCL2*/J_H t(14;18) Translocation Assay: Single Page Guide

Single Amplification Protocol:

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

- *BCL2*/J_H t(14;18) Mbr Mix 1b and
- *BCL2*/J_H t(14;18) mcr Mix 2b

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

- Aliquot volumes are 50 µL for each sample + 150 µL for the positive, negative and no template controls.
- Add an additional 20 µL to correct for pipetting errors.

15.3. Add the appropriate amount of Taq DNA polymerase (0.25 µL @5 U/µL per 55 µL total reaction volume) to each master mix and gently mix by inverting several times or gently vortexing.

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

15.5. Amplify the reactions using the PCR program in Table 4.

15.6. Remove the amplification plate from the thermal cycler.

Nested Amplification Protocol:

15.7. Using gloved hands, remove the 1st round master mixes from the freezer. Allow the tubes to thaw; then gently vortex to mix.

15.8. Follow steps 15.2 - 15.6 using the following master mixes:

- *BCL2*/J_H t(14;18) Mbr Mix 1a and
- *BCL2*/J_H t(14;18) mcr Mix 2a

15.9. Dilute each product from the 1st round reactions 1:100 (2 µL + 198 µL of molecular biology grade distilled water) and mix prior to addition to the 2nd round Master Mixes.

15.10. Using gloved hands, remove the 2nd round master mixes from the freezer. Allow the tubes to thaw; then gently vortex to mix.

15.11. Follow steps 15.2 - 15.6 using the following master mixes:

- *BCL2*/J_H t(14;18) Mbr Mix 1b and
- *BCL2*/J_H t(14;18) mcr Mix 2b

Note: For sample reactions, add the diluted amplification products to the following master mixes:

- *BCL2*/J_H t(14;18) Mbr Mix 1a product added into *BCL2*/J_H t(14;18) Mbr Mix 1b
- *BCL2*/J_H t(14;18) mcr Mix 2a product added into *BCL2*/J_H t(14;18) mcr Mix 2b

Gel Detection – Agarose TBE Gels

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

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

15.14. Load 20 µL of this mixture into separate wells of the gel, and flank with DNA size standards.

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

15.16. Photograph the gel, interpret the data.