Instructions for Use

BCL1/J_H Translocation Assay

RUO

For Identification of BCL1/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	13080010	*BCL1*/J_H Translocation Assay for Gel Detection	33 Reactions
REF	13080020	*BCL1*/J_H Translocation Assay MegaKit for Gel Detection	330 Reactions

Table of Contents

1. Assay Use

This Research Use Only assay identifies *BCL1*/J_H t(11;14) translocations and is useful for the evaluation of new research and methods in malignancy studies.

2. Summary and Explanation of the Test

2.1. Background

This aberrant *BCL1*/J_H t(11;14) translocation juxtaposes genes of the immunoglobulin heavy chain (*IGH*) gene on chromosome 14q32 with the cyclin D1 gene on chromosome 11q13. The juxtaposition of *IGH*-sequences results in the transcriptional activation of cyclin D1.[2,3] Cyclin D1 is involved in the regulation of the G1 progression and G1/S transition of the cell cycle.[8] Translocation does not lead to expression of a fusion protein. In fact, oncogenesis is due to a promoter/enhancer exchange, wherein the immunoglobulin gene enhancer stimulates the expression of cyclin D1. Overexpression of cyclin D1, in turn, accelerates passage of transformed cells through the G1 phase.

2.2. Summary

Invivoscribe's gene rearrangement and translocation assays represent a simple approach to PCR-based clonality testing. These standardized assays were carefully optimized testing positive and negative control samples using multiplex master mixes. Included in this test kit are two (2) master mixes. The *BCL1*/J_H master mix targets the major translocation cluster (MTC) of the *BCL1* locus and the joining region of the Ig heavy chain locus. The other master mix, the Specimen Control Size Ladder, targets multiple genes and generates a series of amplicons of 96, 197, 297, 397 and 602 base pair (bp) that can be used to ensure that the quality and quantity of input DNA is adequate to yield a valid result.

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 MTC region of the *BCL1*/J_H translocation and amplifies genomic DNA between primers that target the *BCL1* gene and the conserved joining (J_H) regions of the *IGH* gene (*BCL1*/J_H Tube master mix). **Breakpoints that occur outside the MTC will not be identified by this particular test**. Therefore, a negative result does not completely exclude the presence of a *BCL1*/J_H gene rearrangement in the sample.[2] DNA from a normal lymphocyte population will also generate a negative result.

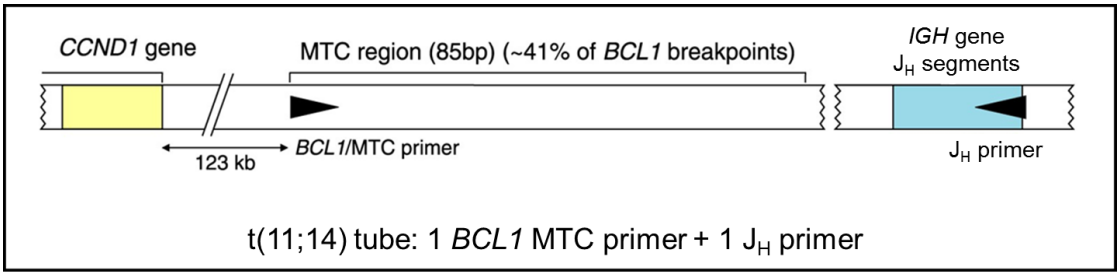

Figure 1. Schematic diagram of the *IGH-CCND1* t(11;14) translocation showing the cyclin D1 (*CCND1*) gene on the left and the Ig heavy chain (*IGH*) gene on the right. Shown are relative positions and orientations for the *BCL1*/MTC primer and the J_H primer, which are included in the BCL1/J_H Master Mix tube.

3.2. Gel Detection

Gel electrophoresis, such as agarose gel electrophoresis or non-denaturing polyacrylamide gel electrophoresis (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.

4. Reagents

4.1. Reagent Components

Table 1. Available Kits

Catalog #		Description	Total Reactions
REF	13080010	*BCL1*/J_H Translocation Assay for Gel Detection	33 Reactions
REF	13080020	*BCL1*/J_H Translocation Assay MegaKit for Gel Detection	330 Reactions

Table 2. Reagent Components

Reagent	Catalog # (REF)	Reagent Components (active ingredients)	Unit of Quantity	13080010 # of Units	13080020 # of Units	Storage Temp.
Master Mixes	23080010	***BCL1*/J_H Tube Master Mix** Oligonucleotides targeting the MTC of the *BCL1* gene and the J region of the *IGH* gene in a buffered salt solution.	1500 µL	1	10	-85°C to -65°C
Template Amplification Control Master Mix	20960020	**Specimen Control Size Ladder Master Mix** Multiple oligonucleotides targeting housekeeping genes.	1500 µL	1	10	
Control and Standard	40880550	**IVS-0010 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
	40920010	**IVS-0000 Polyclonal Control DNA** 200 µg/mL of DNA in 1/10th TE solution	100 µL	1	5	

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

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 comes 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 handle 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 you determine your optimal storage needs.

5. Instruments

5.1. Thermal cycler

- Use or function: Amplification of DNA samples
- Performance characteristics and specification:
  - Minimum Thermal Range: 15ºC to 96ºC
  - Minimum Ramping Speed: 0.8ºC/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

- 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 your institute's Bloodborne Pathogen program and/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 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. Ship at ambient temperature; OR
- Formalin-fixed paraffin embedded tissue or slides.

6.4. Sample Preparation

- Test all samples in **singlicate**.
- Include positive, negative, and no template controls with each master mix tested.

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.

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.

- When testing unknown samples, use a template amplification control master mix (*e.g.*, Amplification Control or Specimen Control Size ladder) 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

See Table 2 for materials provided.

7.2. Materials Required (not provided)

Table 3. Materials Required (not provided)

Reagent/Material	Recommended Reagents/Materials and Suppliers	Part Number	Notes
Ficoll Separation	Thermo Fisher Scientific: • Ficoll Paque PREMIUM • 1X PBS diluted from 10X PBS • RPMI 1640 • Fetal Bovine Serum Sigma: • DMSO Hybri-Max	45-001-752 70011-044 11875-093 16000-0XX* D2650	*size dependent: 036 (100 mL), 044 (500 mL), or 069 (1000 mL)
Density Gradient Medium	Thermo Fisher Scientific®: • Ficoll-PAQUE Premium	45-001-752	N/A
Buffered Saline Solution	Thermo Fisher Scientific: • 1X PBS diluted from 10X PBS	70011-044	N/A
Growth Medium	Thermo Fisher Scientific: • RPMI 1640 with L-glutamine	11875-093	N/A
Dimethyl sulfoxide	Sigma: • DMSO Hybri-Max	D2650	N/A
Fetal Bovine Serum	Thermo Fisher Scientific: • HyClone™ Fetal Bovine Serum (U.S.), Characterized	SH3007103	N/A
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, PTC-200, PTC-220, 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

Table 3. Materials Required (not provided)

Reagent/Material	Recommended Reagents/Materials and Suppliers	Part Number	Notes
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

- Test 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 **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, aliquot the appropriate volume of each master mix into a clean, sterile microfuge tube.

- 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 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

Program for EagleTaq (RECOMMENDED)			
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

Modified program			
Step	**Temperature**	**Duration**	**Cycles**
1	95°C	7 minutes	1
2	94°C	30 seconds	35
3	60°C	45 seconds	
4	72°C	120 seconds	
5	72°C	10 minutes	1
6	15°C	∞	1

7.4.4. Remove the amplified PCR plate from the thermal cycler.

7.5. Detection

Gel Detection – Agarose TBE Gels

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.

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

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

7.5.4. Photograph the gel and interpret the data.

Gel Detection – Polyacrylamide TBE Gels

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

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

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

7.5.7. 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.

7.5.8. Stain the gels in 0.5 µg/mL Ethidium Bromide (in water or 0.5X TBE Buffer) for 5-10 minutes.

7.5.9. Destain the gels 2X in water for 5-10 minutes.

7.5.10. Visualize the gel with UV illumination.

7.5.11. Photograph the gel and interpret the data.

7.6. Quality Control

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

7.7. Recommended Positive Controls

The amplicon sizes listed were determined using an ABI platform or by gel electrophoresis.

Table 5. Recommended positive controls and size of amplified products

Master Mix	Target	Control DNA	Catalog #	Product Size (bp)
***BCL1*/J_H Tube**	MTC of *BCL1* + *IGH* J_H	**Valid Size Range** IVS-0010 Clonal Control DNA	--- 40880550	**150 - 2000** ~200[1], 600
Specimen Control Size Ladder	Multiple Genes	**Valid Size Range** IVS-0000 Polyclonal Control DNA	--- 40920010	**96, 197, 297, 397, 602** 96, 197, 297, 397, 602

Note: The amplicon sizes listed above were determined using a 2% agarose gel.

[1]**Note:** The ~200 bp band is comprised of a 174 bp and a 200 bp band. Both of these bands co-migrate at ~200 bp on an agarose gel.

8. Interpretation and Reporting

This assay is designed for Research Use Only, not intended for use in diagnostic procedures. PCR based testing does not identify 100% of clonal cell populations; therefore, repeat testing by Next Gen Sequencing (NGS) may be advisable to rule out clonality.

8.1. Sample Reporting

Results can be reported as "**Positive**" or "**Negative**" for "**presence of the *BCL1*/J_H t(11;14) Translocation**".

8.1.1. Report samples that fail to amplify with the Specimen Control Size Ladder 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. **All assay controls must be examined prior to interpretation of sample results.** 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 amplifications. Do not continue with the interpretation of results. Prepare fresh master mix and repeat amplification.
  - **Negative**: Continue with the analysis, provided that the polyclonal control is positive with the Specimen Control Size Ladder.

- **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 Specimen Control Size Ladder:
  - **Generates ~96, 197, 297, 397, and 602 bp products:** Please note smaller PCR fragments are preferentially amplified, and it is not unusual for the 602 bp fragment to have a diminished signal or to be missing entirely. Continue with analysis.
  - **Generates no peaks:** Repeat the assay and/or obtain a new specimen.

Note: Under suboptimal conditions a non-specific product of 550 bp is sometimes generated. To discriminate between specific and non-specific products, the negative control DNA will not show this band. If a band is present in the negative control, we then consider the band non-specific.

8.2. Sample Interpretation

Following the acceptance of the controls, interpret the samples as follows: One or two prominent bands within the valid size range are reported as **"Detection of a *BCL1*/J_H t(11;14) 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, it is important to ignore peaks that occur outside of the valid size range for each master mix. Peaks that are outside of the range cannot be assumed to be valid.

Table 6. Expected size of amplified products

Master Mix	Target	Control DNA	Catalog #	Product Size (bp)
***BCL1*/J_H Tube**	MTC of *BCL1* + *IGH* J_H	**Valid Size Range** IVS-0000 Polyclonal Control DNA IVS-0010 Clonal Control DNA Test samples	--- 40920010 40880550 ---	**150 - 2000** no product ~200[1], 600 ~550[2]
Specimen Control Size Ladder	Multiple Genes	**Valid Size Range** IVS-0000 Polyclonal Control DNA	--- 40920010	**96, 197, 297, 397, 602** 96, 197, 297, 397, 602

Note: The amplicon sizes listed above were determined using a 2% agarose gel.
[1]**Note:** The ~200 bp band is comprised of a 174 bp and a 200 bp band. Both of these bands co-migrate at ~200 bp on an agarose gel.
[2]**Note:** The ~550 bp band is non-specific and may not be observed.

10.2. Sample Data

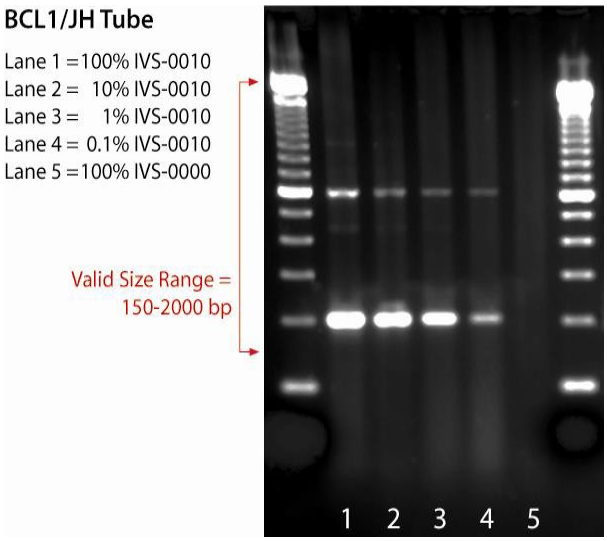

Figure 2. The data shown above were generated with the *BCL1*/J_H Tube master mix.

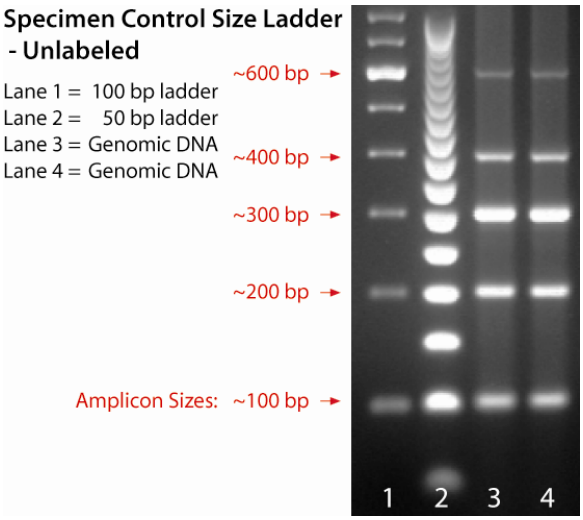

Figure 3. The data shown above were generated with the Specimen Control Size Ladder master mix.

11. Technical and Customer Service

Thank you for purchasing our ***BCL1*/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.,* (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(**12**):2257-2317.
2. Janssen, J.W.G., *et. al.,* (2000). Concurrent activation of a novel putative transforming gene, myeov and cyclin D1 in a subset of multiple myeloma cell lines with t(11;14)(q13:q32). *Blood.* **95**:2691-2698.
3. Shimazaki C, *et. al.,* (1997). Over expression of PRAD1/cyclin D1 in plasma cell leukemia with t(11;14)(q13;q32). *Int J Hematol.* 66(**1**):111-5.

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. *BCL1*/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 master mix).

- Aliquot volumes are 45 µL for each sample + 135 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 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 thermal cycler program below.

Program for EagleTaq (RECOMMENDED)			
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

Modified program			
Step	**Temperature**	**Duration**	**Cycles**
1	95°C	**7 minutes**	1
2	94°C	30 seconds	35
3	60°C	45 seconds	
4	72°C	**120 seconds**	
5	72°C	10 minutes	1
6	15°C	∞	1

Gel Detection – Agarose TBE Gels

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

15.8. Individually mix 20 µL from each amplified PCR product 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 the gel and interpret the 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. Voltage and run time can be adapted accordingly.

15.16. Stain the gels in 0.5 µg/mL Ethidium Bromide (in water or 0.5X TBE Buffer) for 5-10 minutes.

15.17. Destain the gels 2X in water for 5-10 minutes.

15.18. Use UV illumination for visualization.

15.19. Photograph the gel and interpret the data.