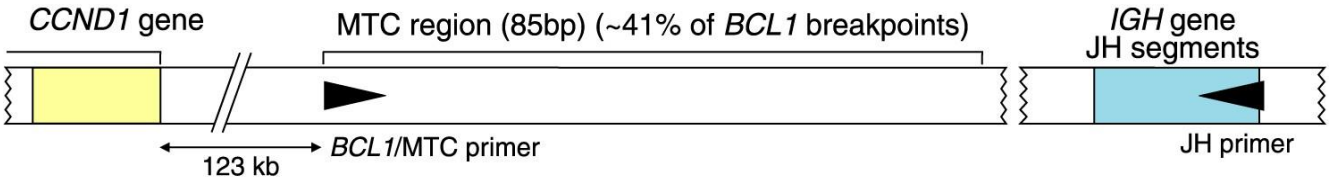
Instructions for Use

BCL1/JH Translocation Assay

For Identification of Mantle Cell and other Lymphomas and Leukemias

For RESEARCH USE ONLY. Not for use in diagnostic procedures.



t(11;14) tube: 1 *BCL1* MTC primer + 1 JH primer

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Manufactured in U.S.A.

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

Catalog #	Products	Quantity
REF 1-308-0010	*BCL1*/JH Translocation Assay for Gel Detection	33 Reactions
REF 1-308-0011	*BCL1*/JH Translocation Assay for ABI Fluorescence Detection	33 Reactions
REF 1-308-0020	*BCL1*/JH Translocation Assay MegaKit for Gel Detection	330 Reactions
REF 1-308-0021	*BCL1*/JH Translocation Assay MegaKit for ABI Fluorescence Detection	330 Reactions

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Thank you for purchasing our ***BCL1*/JH 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.** Questions received during business hours usually receive a response within an hour. Alternatively, you can call for technical assistance and for information on our testing kits at (858) 224-6600 between the hours of 8:00 AM and 5:00 PM Pacific Standard Time.

1. Notice

This product is covered by one or more of the following patents and patent applications owned by or exclusively licensed to Invivoscribe Technologies, Inc. (IVS): United States Patent Application Number 10/531,106, European Patent Number EP 1549764B1 and other pending patent applications originating from European Patent Application Numbers 03756746.8 (16 countries) and Japanese Patent Number JP04708029B2.

Use of this product may require nucleic acid amplification methods such as Polymerase Chain Reaction (PCR). Any necessary license to practice amplification methods or to use amplification enzymes or equipment covered by third party patents is the responsibility of the user and no such license is granted by Invivoscribe Technologies, Inc., expressly or by implication. This product is sold **FOR RESEARCH USE ONLY; not for use in diagnostic procedures**.
This assay is based on the EuroClonality/BIOMED-2 Concerted Action BMH4-CT98-3936.

2. Principle

NOTICE: Invivoscribe Technologies' Gene Rearrangement and Translocation 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 testing more than 400 clinical samples using Revised European/American Lymphoma (REAL) Classification. Testing was done 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 appear in a leading peer-reviewed journal, Leukemia. 2003 Dec;17(12):2257-2317 (Nature Publishing Group).

BACKGROUND:
The t(11;14)(q13;q32) is mainly found in mantle cell lymphoma, but has also seen in B-prolymphocytic leukaemia (10-20%), plasma cell leukaemia, splenic lymphoma with villous lymphocytes, chronic lymphocytic leukaemia (2-5%) and in multiple myeloma (20-25%). (Huret JL. t(11;14)(q13;q32). Atlas Genet. Cytogenet. Oncol. Haematol. May 1998). Fluorescence-in-situ-hybridization (FISH) with probes flanking the *BCL1* translocation breakpoint cluster region at chromosome 11 band q13, revealed that all mantle cell lymphomas (as defined by the REAL-classification) carry the t(11;14)(q13;q32) (Coignet 1996; Vaandrager 1996). The breakpoints are scared over a region of 350-kb and ~41% of these breakpoint subcluster in a locus of only 1-kb referred to as the *BCL1*-major-translocation-cluster, the *BCL1*-MTC, region (Vaandrager 1996).

This aberrant gene rearrangement 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 (De Boer Oncogene 1995; De Boer Blood 1995). Cyclin D1 is involved in the regulation of the G1 progression and G1/S transition of the cell cycle. 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. In the revised WHO-classification the presence of the t(11;14)(q13;q32) and/or overexpression of cyclin D1 as added as one of the characteristics for mantle cell lymphoma.

The ~41% of breakpoints clustered in the 1-kb *BCL1*/MTC locus can be detected by PCR methodology. However, it should be underlined that breakpoints that occur outside the *BCL1*-MTC locus will not be identified by this particular test. Therefore, a negative result does not completely exclude the presence of a *BCL1*/JH gene rearrangement in the sample. Results of this test must always be interpreted in the context of morphologic and other relevant data and should not be used alone for a diagnosis of malignancy.

This test is most useful when confronted with a difficult differential diagnosis that includes mantle cell lymphoma. For instance, a neoplastic B-cell proliferation in tissue, blood or bone marrow that is difficult to categorize as chronic lymphocytic leukemia/small lymphocytic lymphoma, follicular lymphoma, lymphoma of mucosa-associated lymphoid tissue or mantle cell lymphoma could readily be classified as the latter if a *BCL1*/JH gene rearrangement were detected.

This has important clinical implications, since mantle cell lymphomas are typically more aggressive and have an overall worse prognosis than other low-grade B-cell lymphomas. Since this molecular abnormality is also a tumor-specific marker, it can be used for staging purposes and to monitor patients for disease relapse after treatment, if their original lymphoma was studied and shown to have a *BCL1*/JH gene rearrangement.

Included in this test kit are two master mixes. The *BCL1*/JH 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 100, 200, 300, 400 and 600 base pairs to ensure that the quality and quantity of input DNA is adequate to yield a valid result. These robust Invivoscribe assays can be used to test DNA extracted from virtually any source.

3. Assay Uses

***BCL1*/JH t(11;14) Translocation Assays are useful for the study of:**

- Identifying *BCL1/JH* gene rearrangements highly suggestive of mantle cell lymphoma
- Lineage determination of leukemias and lymphomas
- Monitoring and evaluation of disease recurrence
- Detection and assessment of residual disease
- Evaluation of new research and methods in malignancy studies

4. Specimen Requirements

- **This assay tests genomic DNA**

1. 5 cc of peripheral blood, bone marrow biopsy or bone marrow aspirate anti-coagulated with heparin or EDTA. Ship at ambient temperature; OR
2. Minimum 5 mm cube of tissue shipped frozen; or at room temperature or on ice in RPMI 1640; OR
3. 2 µg of genomic DNA; OR
4. Formalin-fixed paraffin embedded tissue or slides.

5. Kit Contents

Controls and Standards	IVS Catalog #	Concentration
IVS-0010 Clonal Control DNA	4-088-0550	100 µL @200 ⌈γ/mL
IVS-0000 Polyclonal Control DNA	4-092-0010	100 µL @200 ⌈γ/mL

Master Mixes	IVS Catalog #	Target
BCL1/JH Tube	2-308-001X	MTC of *BCL1* + *IgH* JH
Specimen Control Size Ladder	2-096-002X	Multiple Genes

Note: **X = Detection format code**

Note: MegaKits contain 10 units of each master mix and 5 units of each Controls and Standards

STATEMENT OF WARNINGS

The assay kit has been optimized to be used 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. Close adherence to the protocol will assure optimal performance and reproducibility. It is recommended that glass distilled de-ionized molecular biology grade water be used with the preparation of specimen DNA. This can be purchased from several manufacturers. In addition, laboratory personnel are reminded to wear appropriate personal protective equipment and follow good laboratory practices and universal precautions when working with specimens. Specimens should be handled in approved biological safety containment facilities and opened only in certified biological safety cabinets. Please see Section 9 for further details.

6. Storage Conditions

PCR master mixes are sensitive to freeze/thaw cycles. Therefore, for any duration other than immediate use, our **master mixes and assay kits should be stored at -65°C to -85°C**.

The reason for this is quite straightforward: Due to the high salt concentrations in our master mixes, the effective freezing and thawing temperature of the master mixes is approximately –10°C. The temperature in a standard laboratory –20°C freezer can easily reach –10°C or warmer during the day when these freezers are opened on a regular basis. At these temperatures, PCR master mixes may go through multiple freeze/thaw cycles, resulting in precipitation of the primers. Accordingly, to minimize the exposure of your master mixes to freeze/thaw cycles, **IVS recommends that master mixes be stored at -65°C to -85°C**.

Please note that our DNA standards are best stored at 2°C to 8°C. However, these standards can be stored at any lower temperature as long as they are vortexed after thawing and before use to ensure that they are re-suspended completely

7. Reagents Required But Not Included

PCR Amplification

AmpliTaq Gold DNA Polymerase or equivalent **(RECOMMENDED)**	(Life Technologies, Cat# N808-0241)
EagleTaq DNA Polymerase or equivalent **(RECOMMENDED)**	(Roche Cat# 05206944190)
AmpliTaq DNA Polymerase	(Life Technologies, Cat# N808-0161)

ABI Fluorescence Detection

HI-DI Formamide with ROX size standards - ABI 310	(IVS, Cat# 6-098-0051)
HI-DI Formamide with ROX size standards - ABI 3100	(IVS, Cat# 6-098-0061)

8. Recommended Positive Controls

Master Mix	Target	Color	Control DNA	Cat#	Product Size (bp)
BCL1/JH Tube	MTC of *BCL1/IGH* JH	**Blue**	**Valid Size Range:** IVS-0010 Clonal Control DNA	--- 4-088-0550	**150-2000** ~200[1], 600
Specimen Control Size Ladder	Multiple Genes	**Blue**	**Valid Size Range** IVS-0000 Polyclonal Control DNA	--- 4-092-0010	**100, 200, 300, 400, 600** 100, 200, 300, 400, 600

Note: The amplicon sizes listed above were determined using a 2% Agarose Gel.

Note[1] 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.

9. Procedure Notes

- Autoclaving does not eliminate DNA contamination.
- Work flow in the PCR laboratory should always be in a one way direction between separate work areas; beginning in Master Mix Preparation, moving to the Specimen Preparation, then to the Amplification and finally to Detection.

1. Do not bring amplified DNA into the areas designated for master mix or specimen preparation.
2. Due to the analytical sensitivity of this test, extreme care should be taken to avoid the contamination of reagents or amplification mixtures with samples, controls or amplified materials. All reagents should be closely monitored for signs of contamination (e.g., negative controls giving positive signals). Discard reagents suspected of contamination.
3. All pipettes, pipet tips and any equipment used in a particular area should be dedicated to and kept to that area of the laboratory.
4. PCR trays, bases and retainers must to be decontaminated in 10% bleach and rinsed with distilled water two separate times before returning them to the starting areas.
5. Sterile, disposable plastic ware should be used whenever possible to avoid RNase or cross-contamination.

10. Reagent Preparation

- All unknown samples should be tested using the template amplification control (**Amplification Control or Specimen Control Size ladder)** master mix. This is to ensure that no inhibitors of amplification are present and there is DNA of sufficient quality and quantity to generate a valid result.
- All samples should be tested in **singlicate**.
- **Positive**, **negative** and **no template** controls should be tested for each of the master mixes.

1. Using gloved hands, remove the master mixes from the freezer. Allow the tubes to thaw; then gently vortex to mix.
2. In 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 should be 45 µL for each sample + 135 µL (3 x 45 µL) for the positive, negative and no template controls. We recommend adding an additional 20 µL to correct for pipetting errors.
3. Add the appropriate amount of either AmpliTaq Gold, EagleTaq or AmpliTaq DNA polymerase (0.25 µL of either AmpliTaq Gold, EagleTaq or AmpliTaq @5 U/µL per 50 µL total reaction volume) to each of the master mixes 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.

11. Sample Preparation

Using any method of DNA extraction, extract the genomic DNA from unknown samples. 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. Testing sample DNAs with the Amplification Control or Specimen Control Size Ladder master mix will ensure that DNA of sufficient quality and quantity was present to yield a valid result.

12. Amplification

1. Aliquot 45 µL of the master mix/enzyme solutions into individual PCR wells or tubes.
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. Amplify the reactions using the following PCR program

We recommend the MJ Research PTC-100, PTC-200 or the PE 2600, 9600 or 9700 thermal cyclers, using the following PCR parameters for the amplifications:

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

(RECOMMENDED) **Standard Program for AmpliTaq Gold** **Or EagleTaq**	Modified Program for AmpliTaq
Step 1: 95°C for 7 minutes	Step 1: 95°C for 7 minutes
Step 2: 95°C for 45 seconds	Step 2: 94°C for 30 seconds
Step 3: 60°C for 45 seconds	Step 3: 60°C for 45 seconds
Step 4: 72°C for **90 seconds**	Step 4: 72°C for **120 seconds**
Step 5: Go to step 2; 34 more times	Step 5: Go to step 2; 34 more times
Step 6: 72°C for 10 minutes	Step 6: 72°C for 10 minutes
Step 7: 15°C forever	Step 7: 15°C forever

Remove the amplification plate from the thermal cycler

13. Detection

- **Not all detection formats are available for all assays**

Available Template Amplification Controls

- The **Amplification Control** master mix primers are labeled with a fluorescent dye (6-FAM). This label is detected as **BLUE** using the differential fluorescence software. The amplicons produced with this master mix are at 235 base pairs. The products of this master mix should be run separately.
- The **Specimen Control Size Ladder** master mix primers are labeled with a fluorescent dye (6-FAM). This label is detected as **BLUE** using the differential fluorescence software. The amplicons produced with this master mix are at ~100, 200, 300, 400 and 600 base pairs. Please note that the ~100 bp band is comprised of 84 bp and 96 bp bands. Both of these bands co-migrate on a gel. The products of this master mix should be run separately.

Gel Detection – Agarose TBE Gels (RECOMMENDED)

1. A 2% MetaPhor or NuSieve 3:1 agarose/TBE gel is prepared.
2. 20 µL from each of the amplification reactions are individually mixed with 4µl of 6X gel loading buffer. 20 µL of this mixture is loaded into separate wells of the gel, flanked by DNA size standards. Products are detected using ethidium bromide or an equivalent dye.
3. Gel is photographed and data are interpreted.

Gel Detection – Polyacrylamide TBE Gels

1. Assemble electrophoresis unit using a 6% non-denaturing polyacrylamide TBE gel (made with 1X TBE, Invitrogen Cat# EC62652Box) and 0.5X TBE running buffer (Invitrogen 5X TBE Cat# LC6675).
2. Add 5 µL of ice-cold non-denaturing bromophenol blue loading buffer to samples.
3. Load 20 µL of mixture into wells of the gel.
4. 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.
5. Gels are stained in 0.5 µg/mL EtBr (in water or 0.5X TBE Buffer) for 5-10 minutes.
6. Gels are destained 2X in water for 5-10 minutes.
7. UV illumination is used for visualization.
8. Gel is photographed and data are interpreted.

Gel Detection – Heteroduplex Analysis

1. Denature 20 µL of PCR products at 94ºC for 5 minutes.
2. Re-anneal PCR products at 4ºC for 60 minutes.
3. Assemble electrophoresis unit using a 6% non-denaturing polyacrylamide TBE gel (made with 1X TBE, Invitrogen Cat# EC62652Box) and 0.5X TBE running buffer (Invitrogen 5X TBE Cat# LC6675).
4. Add 5 µL of ice-cold non-denaturing bromophenol blue loading buffer to samples
5. Load 20 µL of mixture into wells of the gel.
6. 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. Gels are stained in 0.5 µg/mL EtBr (in water or 0.5X TBE Buffer) for 5-10 minutes.
8. Gels are destained 2X in water for 5-10 minutes.
9. UV illumination is used for visualization.
10. Gel is photographed and data are interpreted.

ABI Fluorescence Detection with ABI 310 & 3100 instruments

1. Add 1 µLof reaction products from the *BCL1*/JH Tube in a separate tube and add 10 µL of HI-Deionized Formamide containing ROX size standards to the tube. Mix well.
2. Add 1 µLof reaction product from the template amplification control in a tube and add 10µl of HI-Deionized Formamide containing ROX size standards. Mix well.
3. Reaction products are heated to 95ºC for 2 minutes then snap chilled on ice for 5 minutes.
4. A **sample sheet** and **injection list** is made up for the samples. As the samples are run on the machine, they are fractionated, detected and analyzed by the instrument. Runs are 20-24 minutes in duration. The 310 & 3100 capillary electrophoresis instruments routinely handle 2 runs per hour (48 and 768 samples per day, respectively) and automatically analyze and store data for review or comparison with other test results.
5. Data are automatically displayed as size and color specific peaks. Review profile and controls, report results.

ABI Fluorescence Detection with ABI 373 & 377 instruments

1. PCR Product Dilution: Initially dilute samples 1:10 in HI-Deionized Formamide or water (can be altered if the fluorescence signal is outside the optimal range).
2. Add 2 µLof diluted reaction product from the *BCL1*/JH Tube in a tube + 2 µLof HI-Deionized Formamide containing + 0.5 µL of ROX size standards + 0.5 µLof blue Dextran loading dye. Mix well.
3. Add 2 µL of diluted reaction product from the template amplification control in a tube + 2 µLof HI-Deionized Formamide containing + 0.5 µL ROX size standards + 0.5 µL blue Dextran loading dye. Mix well.
4. Reaction products are heated to 94ºC for 2 minutes then snap chilled on ice for 5 minutes.
5. Load 5 µL of each of these preparations in separate wells of a preheated gel and run using the standard sequencing protocol.

14. Interpretation and Reporting

Note: This assay is for research use only. Although positive results are highly suggestive of malignancy, these assays are designed for Research Use Only and, if used in a clinical setting, should only be used in support of diagnosis. Positive and negative results should be interpreted in the context of all clinical information and laboratory test results. PCR based testing does not identify 100% of clonal cell populations; therefore, repeat testing by Southern blot may be advisable to rule out clonality.

The size range for each of the master mixes has been determined testing positive control samples. For accurate and meaningful interpretation it is important to ignore peaks that occur outside of the proscribed/valid size range for each of the master mixes. Peaks that are outside of the range cannot be assumed to be valid.

Note: "Color" indicates the color of products generated with the master mix when using differential fluorescence detection format (e.g., ABI instruments).

Expected Size of Amplified Products

Master Mix	Target	Color	Control DNA	Cat#	Product Size (bp)
BCL1/JH Tube	MTC of *BCL1/IGH* JH	**Blue**	**Valid Size Range:** IVS-0000 Polyclonal Control DNA IVS-0010 Clonal Control DNA Test Samples	--- 4-092-0010 4-088-0550 ---	**150-2000** No Product ~200[1], 600 ~550[2]
Specimen Control Size Ladder	Multiple Genes	**Blue**	Any Human DNA	---	100, 200, 300, 400, 600

Note: The amplicon sizes listed above were determined using a 2% Agarose Gel.
Note[1] 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.
Note[2] The ~550 bp (weak) non-specific band may be seen.

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

1. Samples that fail to amplify with the Specimen Control Size Ladder 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**".
2. It is acceptable to call a sample "**Positive**" when a translocation product is generated yet the positive control for that master mix fails.
3. Samples that test negative should be repeated if the positive control reaction failed.
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 should not be interpreted**.

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

1. **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.

2. **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 unless specimen tests positive.

3. **Specimen Control Size Ladder**: (This is run on unknown samples only). If the amplification control is:
   - **Positive**: ~100, 200, 300, 400 and 600 base pair products are seen. Because smaller PCR fragments are preferentially amplified, it is not unusual for the 600 base pair fragment to have a diminished signal or to be missing entirely. Continue with analysis.
   - **Negative**: Repeat assay unless specimen tests positive.

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

Sample Interpretation

Following the acceptance of the controls, the clinical samples are interpreted as follows: One or two prominent bands within the valid size range are reported as **"Detection of a *BCL1*/JH t(11;14) Translocation"**

15. Limitations of Procedure

The assay is subject to interference by degradation of DNA or inhibition of PCR due to heparin or other agents. The assay cannot reliably detect less than 1 positive cell per 100 normal cells.

16. References

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17. Appendix

Reagents and Special Supplies

Ficoll Separation

Ficoll-Hypaque or Ficoll-Paque	(Pharmacia, Cat# 17-0840-02)
1X PBS diluted from 10X PBS	(Gibco/BRL, Cat# 70011-044)
RPMI 1640	(Gibco/BRL, Cat# 11875-093)
DMSO Hybri-Max	(Sigma, Cat# D2650)
Fetal Bovine Serum	(Hyclone, Cat# SH30071.03)

Gel Electrophoresis

MetaPhor Agarose, 125g	(Cambrex, Cat# 50180)
NuSieve 3:1 Agarose, 125g	(Cambrex, Cat# 50090)
UltraPure™ 10 mg/ml Ethidium Bromide	(Invitrogen, Cat# 15585-011)
10X BlueJuice™ Gel Loading Buffer	(Invitrogen, Cat# 10816-015)
Ready-Load™ 100 bp Ladder	(Invitrogen, Cat# 10380-012)
Novex® TBE gels (6%, 12 well)	(Invitrogen, Cat# EC62652Box)
Novex® TBE Running Buffer (5X)	(Invitrogen, Cat# LC6675)
Novex® Hi-Density TBE Sample Buffer (5X)	(Invitrogen, Cat# LC6678)

Differential Fluorescence Detection

HI-DI Formamide with ROX size standards - ABI 310	(IVS, Cat# 6-098-0051)
HI-DI Formamide with ROX size standards - ABI 3100	(IVS, Cat# 6-098-0061)
HI-Deionized Formamide	(IVS, Cat# 6-098-0041)
HI-Deionized Formamide	(Applied Biosystems, Cat# 4311320)
GS ROX 50-400HD Size Standard	(Applied Biosystems, Cat# 402985)

18. Troubleshooting Guide

Our laboratories are located in San Diego, California. Technical assistance is most rapidly obtained using our Internet site: **http://www.invivoscribe.com** or by sending an email inquiry to: **support@invivoscribe.com**. Alternatively, you can call (858) 224-6600 for technical assistance and information on our testing kits between the hours of 8:00 AM and 5:00 PM Pacific Standard Time.

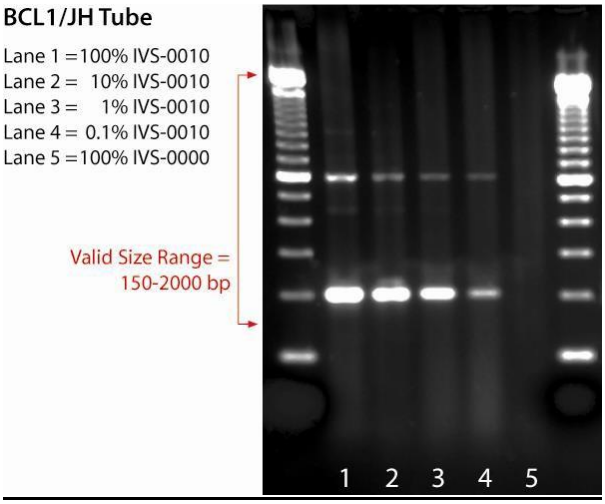
Questions received during business hours usually receive a response within an hour.

19. Sample Data

Figure 1. Gel Detection

Amplified products were amplified using the *BCL1*/JH Tube master mix then run on a 2% agarose gel.

- Lane 1 displays data generated testing the 100% IVS-0010 clonal control DNA.
- Lane 2 displays data generated testing the 10% IVS-0010 clonal control DNA.
- Lane 3 displays data generated testing the 1% IVS-0010 clonal control DNA
- Lane 4 displays data generated testing the 0.1% IVS-0010 clonal control DNA
- Lane 5 displays data generated testing the IVS-0000 Polyclonal Control DNA.



20. *BCL1*/JH Translocation Assay: Single Page Flow Chart

1. Using gloved hands, remove the master mixes from the freezer. Allow the tubes to thaw; then gently vortex to mix.
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 should be 45 µL for each sample + 135 µL for the positive, negative and no template controls. We recommend adding an additional 20 µL to correct for pipetting errors.
3. Add the appropriate amount of either AmpliTaq Gold, EagleTaq or AmpliTaq DNA polymerase (0.25 µL of either AmpliTaq Gold, EagleTaq or AmpliTaq @5 U/µL per 50 µL total reaction volume) to each of the master mixes and gently mix by inverting several times or gently vortexing.
4. Aliquot 45 µL of master mix to individual wells of a PCR plate.
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. Amplify target DNA using the universal thermal cycler program.

Gel Detection – Agarose TBE Gels

1. A 2% MetaPhor or NuSieve 3:1 agarose/TBE gel is prepared.
2. 20 µL from each of the amplification reactions are individually mixed with 4µl of 6X gel loading buffer. 20 µL of this mixture is loaded into separate wells of the gel, flanked by DNA size standards. Products are detected using ethidium bromide or an equivalent dye.
3. Gel is photographed and data are interpreted.

ABI Fluorescence Detection with ABI 310 & 3100 instruments

1. Add 1 µLof reaction products from the *BCL1*/JH Tube in a separate tube and add 10 µL of HI-Deionized Formamide containing ROX size standards to the tube. Mix well.
2. Add 1 µLof reaction product from the template amplification control in a tube and add 10µl of HI-Deionized Formamide containing ROX size standards. Mix well.
3. Reaction products are heated to 95ºC for 2 minutes then snap chilled on ice for 5 minutes.
4. A **sample sheet** and **injection list** is made up for the samples. As the samples are run on the machine, they are fractionated, detected and analyzed by the instrument. Runs are 20-24 minutes in duration. The 310 & 3100 capillary electrophoresis instruments routinely handle 2 runs per hour (48 and 768 samples per day, respectively) and automatically analyze and store data for review or comparison with other test results.
5. Data are automatically displayed as size and color specific peaks. Review profile and controls, report results.