Instructions for Use

BCR/ABL t(9;22) Translocation Assay

RUO

RT-PCR Assay for identification of *BCR/ABL* 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	13100010	*BCR/ABL* t(9;22) Translocation Assay for Gel Detection	30 Reactions
REF	13100031	*BCR/ABL* t(9;22) Translocation Assay for ABI Fluorescence Detection	30 Reactions

Table of Contents

1. Assay Use

This Research Use Only assay identifies *BCR/ABL* t(9;22) translocations.

2. Summary and Explanation of the Test

2.1. Summary

BCR/ABL translocations are associated with a variety of hematologic malignancies. The Philadelphia chromosome (Ph1) is a specific chromosomal abnormality that results from reciprocal t(9;22)(q34;ql1) chromosomal rearrangements fuse coding regions of the *BCR* gene, located on chromosome 22, with the ABL receptor-independent tyrosine kinase gene on chromosome 9. This assay detects and identifies the variety of p190-, p210- and p230-type transcripts produced from all known *BCR/ABL* translocations.

2.2. Background

The master mixes are included in these assay kits used to amplify complementary DNA (cDNA) produced from specimen(s), and positive and negative RNA controls (included). Primers target an internal control transcript (*Abl*) and p190-, p210-, and p230-type transcripts expressed from *BCR-ABL1* translocations. Amplicon products can be analyzed by capillary electrophoresis or standard gel electrophoresis with ethidium bromide staining. This assay is compatible with all standard RNA extraction and cDNA synthesis methods.

3. Principles of the Procedure

3.1. Reverse Transcription - Polymerase Chain Reaction (RT-PCR)

RT-PCR assays are routinely used for the identification of chromosome translocations. This assay targets transcripts expressed from *BCR-ABL1* translocations (see Figure 1). A *BCR-ABL1* translocation is indicated if just one of the 2nd round master mixes (Mix 2b, Mix 2c, Mix 3b, Mix 3c, or Mix 3d) generates product(s) of the valid size.

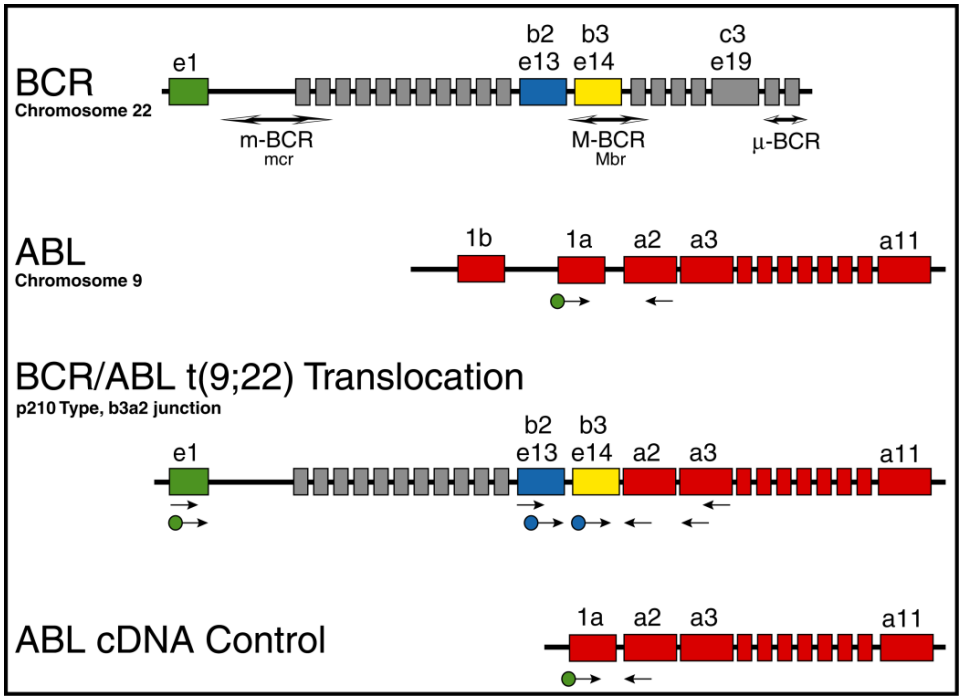

Figure 1. This figure shows the genomic organization of the *BCR* and *ABL* genes on chromosomes 22 and 9, respectively. Boxes represent exon regions of the *ABL* (red boxes) and *BCR* encoding exons (other colors). The solid black line represents intron regions, which have been left incompletely spliced to assist in demarcation of the exon segments. The location of exon regions targeted by labeled and unlabeled primers are indicated by arrows. A p210-type *BCR-ABL1* translocation (b3a2 junction) is depicted in the lower portion of the figure along with the control *ABL* transcript control.

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.

3.3. Differential Fluorescence Detection

A powerful alternative method is use of differential fluorescence detection with primers conjugated with fluorescent dyes that correspond to different targeted regions. Reaction products from several different master mixes can be pooled, fractionated using capillary electrophoresis and detected simultaneously. This detection system results in unsurpassed sensitivity, resolution, differential product detection and quantification. In addition, the laboratory can eliminate the use of agarose and polyacrylamide gels, as well as the use of carcinogens such as ethidium bromide.

4. Reagents

4.1. Reagent Components

Table 1. Available Kits

Catalog #	Description	Total Reactions
REF 13100010	*BCR/ABL* t(9;22) Translocation Assay for Gel Detection	30 Reactions
REF 13100031	*BCR/ABL* t(9;22) Translocation Assay for ABI Fluorescence Detection	30 Reactions

Reagent	Catalog #	Reagent Components (active ingredients)	Unit Quantity	# of Units	Storage Temp.
Controls and Standard	40892800	**IVS-0032 Clonal Control RNA** 400 µg/mL of RNA in water	100 µL	1	-85°C -65°C
	40890910	**IVS-0011 Clonal Control RNA** 400 µg/mL of RNA in water	100 µL	1	
	40893070	**IVS-0035 Clonal Control RNA** 400 µg/mL of RNA in water	100 µL	1	
1st Round Master Mixes	23100010	***BCR/ABL* t(9;22) Mix 1a - Unlabeled** Multiple oligonucleotides targeting the *Abl* gene, serving as an internal control, in a buffered salt solution.	1500 µL	1	-85°C -65°C
	23100020	***BCR/ABL* t(9;22) Mix 2a - Unlabeled** Multiple oligonucleotides targeting all p190-type *BCR/ABL* transcripts in a buffered salt solution.	1500 µL	1	
	23100030	***BCR/ABL* t(9;22) Mix 3a - Unlabeled** Multiple oligonucleotides targeting all p210- and p230-type *BCR/ABL* transcripts in a buffered salt solution.	1500 µL	1	
Gel Detection					
2nd Round Master Mixes	23100040	***BCR/ABL* t(9;22) Mix 1b - Unlabeled** Multiple oligonucleotides targeting the *Abl* gene, serving as an internal control, in a buffered salt solution.	1500 µL	1	-85°C -65°C

Reagent	Catalog #	Reagent Components (active ingredients)	Unit Quantity	# of Units	Storage Temp.
2nd Round Master Mixes	23100050	***BCR/ABL* t(9;22) Mix 2b - Unlabeled** Multiple oligonucleotides targeting all p190-type *BCR/ABL* transcripts in a buffered salt solution.	1500 µL	1	**-85°C -65°C**
	23100060	***BCR/ABL* t(9;22) Mix 2c - Unlabeled** Multiple oligonucleotides targeting all p190-type *BCR/ABL* transcripts in a buffered salt solution.	1500 µL	1	
	23100070	***BCR/ABL* t(9;22) Mix 3b - Unlabeled** Multiple oligonucleotides targeting all p210- and p230-type *BCR/ABL* transcripts in a buffered salt solution.	1500 µL	1	
	23100080	***BCR/ABL* t(9;22) Mix 3c - Unlabeled** Multiple oligonucleotides targeting all p210- and p230-type *BCR/ABL* transcripts in a buffered salt solution.	1500 µL	1	
	23100090	***BCR/ABL* t(9;22) Mix 3d - Unlabeled** Multiple oligonucleotides targeting all p210- and p230-type *BCR/ABL* transcripts in a buffered salt solution.	1500 µL	1	
ABI Fluorescence Detection					
2nd Round Master Mixes	23100041	***BCR/ABL* t(9;22) Mix 1b - HEX** Multiple oligonucleotides targeting the *Abl* gene, serving as an internal control, in a buffered salt solution.	1500 µL	1	**-85°C -65°C**
	23100051	***BCR/ABL* t(9;22) Mix 2b - HEX** Multiple oligonucleotides targeting all p190-type *BCR/ABL* transcripts in a buffered salt solution.	1500 µL	1	
	23100061	***BCR/ABL* t(9;22) Mix 2c - HEX** Multiple oligonucleotides targeting all p190-type *BCR/ABL* transcripts in a buffered salt solution.	1500 µL	1	
	23100071	***BCR/ABL* t(9;22) Mix 3b – 6FAM** Multiple oligonucleotides targeting all p210- and p230-type *BCR/ABL* transcripts in a buffered salt solution.	1500 µL	1	
	23100081	***BCR/ABL* t(9;22) Mix 3c – 6FAM** Multiple oligonucleotides targeting all p210- and p230-type *BCR/ABL* transcripts in a buffered salt solution.	1500 µL	1	
	23100101	***BCR/ABL* t(9;22) Mix 3d – 6FAM** Multiple oligonucleotides targeting all p210- and p230-type *BCR/ABL* transcripts in a buffered salt solution.	1500 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 eye wear). 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 work flow 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 your 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.

5.4. ABI Capillary Electrophoresis Instruments (for ABI Detection)

- Use or function: Fragment detection and analysis
- Performance characteristics and specification:
  - The following capillary electrophoresis instruments will meet the performance needs for this assay:
    - ABI 310 Genetic Analyzer (1-capillary)
    - ABI 3100 Avant Genetic Analyzer (4-capillaries)
    - ABI 3100 Genetic Analyzer (16-capillaries)
    - ABI 3130 Genetic Analyzer (4-capillaries)
    - ABI 3130xL Genetic Analyzer (16-capillaries)
    - ABI 3500 Genetic Analyzer (8-capillaries)
    - ABI 3500xL Genetic Analyzer (24-capillaries)
- Follow manufacturer's installation, operation, calibration and maintenance procedures.
- The ABI instrument used must be calibrated with appropriate Matrix Standards as outlined in section 7.2. *Materials Required (not provided)*.
- Use the default settings for your polymer and capillary type.
- See section 7.5. *Detection*.

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 complementary DNA (cDNA) template. Please refer to section 6.4. *Sample Preparation* for protocols on extracting RNA and making cDNA. Reagents and supplies for these procedures are not provided in this testing kit; please refer to 7.2. *Materials Required (not provided)* for details.

6.4. Sample Preparation

Test all samples in singlicate and use 24 µL of the control RNAs in cDNA synthesis reactions. The following controls are included in this testing kit:

- IVS-0032 Clonal Control RNA (*BCR/ABL* p190 e1a2 Control RNA)
- IVS-0011 Clonal Control RNA (*BCR/ABL* p210 b3a2 Control RNA)
- IVS-0035 Clonal Control RNA (Negative control for *BCR/ABL*)

Note: The positive control RNA can be used as both positive and extraction controls, if added to a sample during the extraction process. These control RNAs are very labile--to prevent degradation, add to samples after the chaotropic agent is added and the sample is homogenized. RNA STAT-60 is recommended for RNA isolation.

Note: Human peripheral blood and bone marrow are collected in tubes containing EDTA or heparin as an anticoagulant. Heparin is a powerful inhibitor of PCR. However, Ficoll and other separation media are generally effective at removing heparin from samples.

- Amplification controls that test for presence of the housekeeping *ABL* transcript are provided (master mixes 1a and 1b) to detect amplification inhibitors. Use these mixes to test every sample to ensure PCR inhibitors are not present; a 94 bp product is generated from valid sample cDNA.
- Peripheral blood lymphocytes (PBLs) are isolated by diluting the whole blood 1:1 with RPMI 1640 and banding on Ficoll-Hypaque.
- Cells collected at the interface are washed 3x with RPMI or phosphate buffered saline (PBS) before RNA extraction.

6.4.1. Isolate cells using separation media (*e.g.*, Ficoll-Hypaque); rinse using PBS or RPMI 1640.

6.4.2. Extract RNA from unknown samples.

6.4.3. Synthesize cDNA from unknown sample RNAs and the positive and negative control RNAs included in the assay kit.

6.4.4. Carefully add 5 µL of the resultant cDNAs into each of the appropriate reaction master mix aliquots; pipette up and down several times to mix.

6.5. Sample Storage

Store samples using a method that prevents degradation of cDNA.

7. Assay Procedure

7.1. Materials Provided

Table 2. Materials Provided

Gel Detection Kits	
Catalog #	**Description**
REF 23100010	*BCR/ABL* t(9;22) Mix 1a - Unlabeled
REF 23100020	*BCR/ABL* t(9;22) Mix 2a - Unlabeled
REF 23100030	*BCR/ABL* t(9;22) Mix 3a - Unlabeled
REF 23100040	*BCR/ABL* t(9;22) Mix 1b - Unlabeled
REF 23100050	*BCR/ABL* t(9;22) Mix 2b - Unlabeled
REF 23100060	*BCR/ABL* t(9;22) Mix 2c - Unlabeled
REF 23100070	*BCR/ABL* t(9;22) Mix 3b - Unlabeled
REF 23100080	*BCR/ABL* t(9;22) Mix 3c - Unlabeled
REF 23100090	*BCR/ABL* t(9;22) Mix 3d - Unlabeled
REF 40892800	IVS-0032 Clonal Control RNA
REF 40890910	IVS-0011 Clonal Control RNA
REF 40893070	IVS-0035 Clonal Control RNA

ABI Fluorescence Detection Kits	
Catalog #	**Description**
REF 23100010	*BCR/ABL* t(9;22) Mix 1a - Unlabeled
REF 23100020	*BCR/ABL* t(9;22) Mix 2a - Unlabeled
REF 23100030	*BCR/ABL* t(9;22) Mix 3a - Unlabeled
REF 23100041	*BCR/ABL* t(9;22) Mix 1b – HEX
REF 23100051	*BCR/ABL* t(9;22) Mix 2b – HEX
REF 23100061	*BCR/ABL* t(9;22) Mix 2c – HEX
REF 23100071	*BCR/ABL* t(9;22) Mix 3b - 6FAM
REF 23100081	*BCR/ABL* t(9;22) Mix 3c - 6FAM
REF 23100101	*BCR/ABL* t(9;22) Mix 3d - 6FAM
REF 40892800	IVS-0032 Clonal Control RNA
REF 40890910	IVS-0011 Clonal Control RNA
REF 40893070	IVS-0035 Clonal Control RNA

7.2. Materials Required (not provided)

Table 3. Materials Required (not provided)

Reagent/Material	Recommended Reagents/Materials and Suppliers	Part Number	Notes
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
RNA Extraction Reagent	Tel-Test: • RNA STAT-60	CS-110	N/A
Chloroform	Sigma: • Chloroform	C-2432	N/A
Isopropanol	Sigma: • Isopropanol (2-propanol)	I-0398	N/A
Ethanol	Sigma: • 100% Ethanol (Ethyl Alcohol)	27074-1	N/A
RNAse Inhibitor	Ambion: • RNAse ZAP	9780	N/A
Reverse Transcriptase	Thermo Fisher Scientific: • SuperScript II RT, 4x10,000 units	18064-071	N/A
Random Primers	Thermo Fisher Scientific: • Random Primers, 9 units	48190-011	N/A

Table 3. Materials Required (not provided)

Reagent/Material	Recommended Reagents/Materials and Suppliers	Part Number	Notes
dNTPs	Thermo Fisher Scientific: • 20 mM dNTP Stock solution	27-2094-02	N/A
Ribonuclease Inhibitor	Thermo Fisher Scientific: • RNasin® Ribonuclease Inhibitor, 10,000 units	N2115	N/A
Mineral Oil	Sigma: • Mineral Oil Light	M-3516	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 1 µL and 1000 µL.
Thermal cycler	Thermo Fisher Scientific: • Veriti Dx Thermal Cycler Bio-Rad: • MJ Research PTC-100, 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
Gel Detection			
Reagent/Material	**Recommended Reagents/Materials and Suppliers**	**Part Number**	**Notes**
Ethidium Bromide	Thermo Fisher Scientific: • UltraPure™ 10 mg/mL Ethidium Bromide	15585-011	N/A
Agarose Gels	Thermo Fisher Scientific: • NuSieve™ 3:1 Agarose, 125 g • MetaPhor™ Agarose, 125 g	BMA50090 BMA50180	
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
ABI Fluorescence Detection			
ABI Capillary Electrophoresis Instrument	Thermo Fisher Scientific: • ABI 310, 3100, or 3500 series	N/A	N/A
Hi-Di Formamide	Thermo Fisher Scientific: • Hi-Di™ Formamide	4311320	N/A

Table 3. Materials Required (not provided)

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

Size Standards	Invivoscribe:
	• Hi-Di Formamide w/ROX size standards for ABI 3100
	Thermo Fisher Scientific:
	• For ABI 3100 or 3130 instruments:
	  ○ GeneScan™ - 400HD [ROX]™
	• For ABI 3500 instruments:
	  ○ GeneScan™ – 600 [LIZ]™ 2.0
		60980061
		
		402985
		
		4408399
			N/A

Spectral Calibration Dye Sets	Thermo Fisher Scientific:
	• For ABI 3500 instruments:
	  ○ DS-33 Matrix Standard Kit (Dye Set G5)
	• For ABI 3100 instruments:
	  ○ DS-30 Matrix Standard Kit (Dye Set D)
	• For ABI 310 instruments:
	  ○ NED Matrix Standard
	  ○ And Fluorescent Amidite Matrix Standards [6FAM, TET, HEX, TAMRA, ROX]
		4345833
		
		4345827
		
		402996
		
		401546
			Dye set used to spectrally calibrate ABI instrument for use with 6FAM, HEX, NED and ROX

Polymer	Thermo Fisher Scientific:
	• POP-4™ Polymer:
	  ○ POP-4 for 310 Genetic Analyzers
	  ○ POP-4 for 3100/3100-Avant Genetic Analyzers
	  ○ POP-4 for 3130/3130xL Genetic Analyzers
	• POP-7™ Polymer:
	  ○ POP-7 for 3130/3130xL Genetic Analyzers
	  ○ POP-7 for 3500/3500xL Genetic Analyzers
		402838
		4316355
		4352755
		
		4352759
		4393714
			N/A

Buffer	Thermo Fisher Scientific: • 10X Genetic Analyzer Buffer with EDTA	402824	Dilute 1:10 in sterile water before use

7.3. Reagent Preparation

- Test all samples in **singlicate**.
- Test **positive**, **negative** and **no template** controls for 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 a sterile microcentrifuge 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.

7.4. Amplification

1st Round of Amplification Protocol

7.4.1. Thaw the 1st round master mixes:

- *BCR/ABL* t(9;22) Master Mix 1a
- *BCR/ABL* t(9;22) Master Mix 2a
- *BCR/ABL* t(9;22) Master Mix 3a

7.4.2. Transfer the required volumes for testing the samples and controls to separate sterile microcentrifuge tubes (one tube for each master mix).

7.4.3. Add DNA polymerase to these master mixes and gently mix the tube contents.

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

7.4.5. Add 5 µL of sample or control cDNA to the individual tubes or wells containing the respective master mix reactions.

7.4.6. Amplify the reactions using the appropriate 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

7.4.7. Remove the amplification plate from the thermal cycler.

2nd Round of Amplification Protocol

7.4.8. Dilute each product amplified during the 1st round PCR by 1:100 (2 µL + 198 µL of molecular biology grade distilled water) and mix prior to addition to the 2nd round master mixes in step 7.4.12 below.

7.4.9. Thaw the 2nd round master mixes:

- *BCR/ABL* t(9;22) Master Mix 1b
- *BCR/ABL* t(9;22) Master Mix 2b
- *BCR/ABL* t(9;22) Master Mix 2c
- *BCR/ABL* t(9;22) Master Mix 3b
- *BCR/ABL* t(9;22) Master Mix 3c
- *BCR/ABL* t(9;22) Master Mix 3d

7.4.10. Transfer the volumes required for testing the samples and controls to separate sterile microcentrifuge tubes (one tube for each master mix).

7.4.11. Add DNA polymerase to these master mixes and gently mix the tube contents.

7.4.12. Aliquot 50 µL of the master mix/enzyme solutions into individual PCR wells ortubes.

7.4.13. Add 5 µL of the diluted amplification product from step 7.4.8 to the individual tubes or wells containing the respective master mixes.

- *BCR/ABL* t(9;22) Mix 1a product added into *BCR/ABL* t(9;22) Master Mix 1b
- *BCR/ABL* t(9;22) Mix 2a product added into *BCR/ABL* t(9;22) Master Mixes 2b and 2c
- *BCR/ABL* t(9;22) Mix 3a product added into *BCR/ABL* t(9;22) Master Mixes 3b, 3c, and 3d

7.4.14. Amplify the reactions using the same PCR program (see Table 4).

7.5. Detection

- Not all detection formats are available for all assays

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

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

ABI Fluorescence Detection with ABI 310 & 3100 instruments

7.5.13. Add 1 µL of reaction products from the *BCR/ABL* t(9;22) Master Mixes 1b, 2b, 2c, 3b, 3c, and 3d to separate tubes, then add 10 µL of Hi-Deionized Formamide containing ROX size standards to each tube. Mix well.
7.5.14. Heat PCR products to 95ºC for 2 minutes then snap chill on ice for 5 minutes.
7.5.15. Prepare a **sample sheet** and **injection list** for the samples. As the samples are run on the machine, they are fractionated, detected and analyzed by the instrument.
7.5.16. Review profile and controls, report results

- Data are automatically displayed as size and color specific peaks.

ABI Fluorescence Detection with ABI 3500 instruments

Note: Due to instrument to instrument variation in the performance of the ABI 3500 platform, the amount of formamide, sample and size standard listed in the protocol is intended to be a starting point. The protocol may need to be optimized for specific ABI 3500 Platforms.

7.5.17. In a new microcentrifuge tube, mix an appropriate amount (9.5 µL per reaction) of Hi-Di Formamide with LIZ Size Standards.[a] Vortex well.
7.5.18. In a new 96-well PCR plate, add 9.5 µL of Hi-Di Formamide with LIZ size standards to individual wells for each reaction.
7.5.19. Transfer 0.5 µL of each reaction to the wells containing Hi-Di Formamide with LIZ size standards.

- Add only one sample per well.
- Pipette up and down to mix.

7.5.20. Cap or cover the PCR plate.
7.5.21. Heat denature the samples at 95ºC for 3 minutes, then snap chill on ice for 5 minutes.
7.5.22. Prepare a **sample sheet** and **injection list** for the samples.
7.5.23. Run the samples on an ABI 3500 capillary electrophoresis instrument according to its user manual.

- Data are automatically displayed as size and color specific peaks.

7.5.24. Review profile and controls, report results.

[a]**Note:** Please see Applied Biosystems' accompanying product insert for mixing Hi-Di Formamide with size standards for different ABI instruments.

7.6. Recommended Positive Controls

Table 5. Recommended positive controls and size of amplified products

Master Mix	Target	Color	Control RNA	Catalog #	Product Size (bp)
***BCR/ABL* t(9;22) Mix 1a**	Abl	**N/A**	**Valid Size Range** IVS-0035 Clonal Control RNA	--- 40893070	**Specified Rearrangements** ~300
***BCR/ABL* t(9;22) Mix 1b**	Abl	**Green**	**Valid Size Range** IVS-0035 Clonal Control RNA	--- 40893070	**Specified Rearrangements** 94
***BCR/ABL* t(9;22) Mix 2a**	p190	**N/A**	**Valid Size Range** IVS-0032 Clonal Control RNA	--- 40892800	**Specified Rearrangements** ~425
***BCR/ABL* t(9;22) Mix 2b**	p190	**Green**	**Valid Size Range** IVS-0032 Clonal Control RNA	--- 40892800	**Specified Rearrangements** 186
***BCR/ABL* t(9;22) Mix 2c**	p190	**Green**	**Valid Size Range** IVS-0032 Clonal Control RNA	--- 40892800	**Specified Rearrangements** 371
***BCR/ABL* t(9;22) Mix 3a**	p210 & p230	**N/A**	**Valid Size Range** IVS-0011 Clonal Control RNA	--- 40890910	**Specified Rearrangements** ~375
***BCR/ABL* t(9;22) Mix 3b**	p210 & p230	**Blue**	**Valid Size Range** IVS-0011 Clonal Control RNA	--- 40890910	**Specified Rearrangements** 126
***BCR/ABL* t(9;22) Mix 3c**	p210 & p230	**Blue**	**Valid Size Range** IVS-0011 Clonal Control RNA	--- 40890910	**Specified Rearrangements** 311
***BCR/ABL* t(9;22) Mix 3d**	p210 & p230	**Blue**	**Valid Size Range** IVS-0011 Clonal Control RNA	--- 40890910	**Specified Rearrangements** 242

Note: The amplicon sizes listed above were determined using an ABI platform. Amplicon sizes seen on each specific CE instrument may differ 1-4 bp from those listed above depending on the platform of detection (ABI) and the version of the analysis software used. Once identified, the amplicon size as determined on the specific platform used will be consistent from run to run.

8. Interpretation and Reporting

This assay is for research use only; not intended for diagnostic purposes.

8.1. Sample Analysis

Confirm identities of all product peaks by DNA sequencing:

8.1.1. Results are reported as "**positive**" or "**negative**" for the "**detection of *BCR/ABL* t(9;22) transcripts**". Further, the specific *BCR/ABL* junction is identified using this assay and can be reported (see below).

8.1.2. Samples that fail to produce *BCR/ABL* specific amplicons and internal RNA/cDNA control products (94 bp *ABL* amplicon generated using Master Mix 1b) on two successive runs will be reported as "**A result cannot be reported because the mRNA was too degraded for analysis**".

8.1.3. It is acceptable to call "**positive**" samples that give consistent p190, p210 or p230 *BCR/ABL* transcript profiles yet fail to produce the 94 bp *ABL* product using Master Mix 1b.

8.1.4. Assays in which any one of the negative controls has failed will be repeated.

8.2. Control Analysis

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

- **Negative Control:** (water or no template control) If the master mix negative 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.
- **Negative Control:** (*BCR/ABL* negative control RNA; or cDNA from a negative control sample) If the

specimen control is:

- **Positive:** Any product seen in absence of cDNA synthesis indicates possible contamination of extracted RNA. Do not continue with interpretation. Repeat extractions and assay.

Note: A **94 bp *ABL* control product** is generated from normal samples using Master Mixes 1a and 1b.

- **Negative**: Continue with examination of samples.

- **Positive Control:** (Positive control *BCR/ABL* RNAs following cDNA synthesis) If the specimen control is:
  - **Positive:** **p210 positive control sample is expected to display the variety of b3a2-specific peaks** (see below). Continue with analysis.

Note: **Positive** p190 positive control sample will display *BCR* e1a2-specific peaks (see below). Continue with analysis.

  - **Negative:** Repeat assay.

Note: Some *BCR/ABL* samples express numerous *BCR/ABL* transcripts. For example, K562 cells produce both *BCR/ABL* p210 and p190 type RNA transcripts. In addition, a variety of *BCR/ABL*-specific splice-variants can be amplified. Therefore the specific pattern of products will depend to some degree on the concentration of the various *BCR/ABL* transcript RNAs present in an individual sample. However, the specificity and identities of these products have been confirmed by sequencing.

8.3. Sample Interpretation

Following the acceptance of the controls, the samples are interpreted as follows: One or two prominent bands within the valid size range are reported as: "**Detection of *BCR/ABL* t(9;22) translocation."**

9. Limitations of Procedure

- The assay is subject to interference by degradation of the mRNA.
- The assay is subject to interference by degradation of cDNA or inhibition of PCR due to heparin or other agents.
- Stability of specimens for this assay is confirmed targeting a region of the *ABL* transcript. This transcript is an **internal amplification standard**. Presence of the 94 bp amplification product using Master Mix 1b confirms:

1) there was RNA of sufficient quality and quantity to convert to cDNA, and
2) cDNA synthesis progressed from this RNA template in a manner sufficient to provide adequate material for PCR amplification of this control region.

10. Expected Results

Transcript profiles have been established for all of the p190 and p210 *BCR/ABL* products.

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

10.1. Expected Size of Amplified Products

Table 6. Expected Size of Amplified Products

Master Mix	Target	Color	Control RNA	Cat#	Product Size (bp)
***BCR/ABL* t(9;22) Mix 1b**	*Abl*	**Green**	**Valid Size Range** IVS-0032 Clonal Control RNA IVS-0003 Clonal Control RNA IVS-0011 Clonal Control RNA *BCR/ABL* p230 IVS-0035 Clonal Control RNA	--- 40892800 40890190 40890910 --- 40893070	**Specified Rearrangements** 94 94 94 94 94
***BCR/ABL* t(9;22) Mix 2b**	p190	**Green**	**Valid Size Range** IVS-0032 Clonal Control RNA IVS-0011 Clonal Control RNA	--- 40892800 40890910	**Specified Rearrangements** 186[1] 186[1]
***BCR/ABL* t(9;22) Mix 2c**	p190	**Green**	**Valid Size Range** IVS-0032 Clonal Control RNA IVS-0011 Clonal Control RNA	--- 40892800 40890910	**Specified Rearrangements** 371[1] 371[1]

Table 6. Expected Size of Amplified Products

Master Mix	Target	Color	Control RNA	Cat#	Product Size (bp)
***BCR/ABL* t(9;22) Mix 3b**	p210	**Blue**	**Valid Size Range** IVS-0032 Clonal Control RNA IVS-0011 Clonal Control RNA *BCR/ABL* p230	--- 40892800 40890910 ---	**Specified Rearrangements** 200[1] 126, 171[2] 600, 650
***BCR/ABL* t(9;22) Mix 3c**	p210	**Blue**	**Valid Size Range** IVS-0032 Clonal Control RNA IVS-0003 Clonal Control RNA IVS-0011 Clonal Control RNA *BCR/ABL* p230 IVS-0035 Clonal Control RNA	--- 40892800 40890190 40890910 --- 40893070	**Specified Rearrangements** 350[1] 236 311, 356[2] 800 ---[3]
***BCR/ABL* t(9;22) Mix 3d**	p210	**Blue**	**Valid Size Range** IVS-0011 Clonal Control RNA *BCR/ABL* p230	--- 40890910 ---	**Specified Rearrangements** 242 750

Note: The amplicon sizes listed above were determined using an ABI platform. Amplicon sizes seen on each specific CE instrument may differ 1-4 bp from those listed above depending on the platform of detection (ABI, etc.) and the version of the analysis software used. Once identified, the amplicon size as determined on the specific platform used will be consistent from run to run.

Note: Only the most prominent product bands are listed. There are a number of splice variants produced.

[1]**Note:** These products may also be present in samples positive for p210 breakpoints because p190 products are sometimes transcribed by cells with the p210 translocations.

[2]**Note:** These products may not be seen with ABI Fluorescence Detection.

[3]**Note:** Products below 120 bp may be present in this negative control or in samples; however these should not be interpreted as positive samples.

10.2. Sample Data

Gel Detection: The data shown in Figure 2 - Figure 7 were generated using the master mixes indicated. *BCR/ABL* t(9;22) Master Mixes 3a, 3b, and 3c target the *BCR/ABL* p210- and p230-type translocations. IVS-0011 exhibits the p210 b3a2 product and amplifies with all 3 master mixes, whereas IVS-0003 exhibits the p210 b2a2 product and only amplifies with Mix 3c.

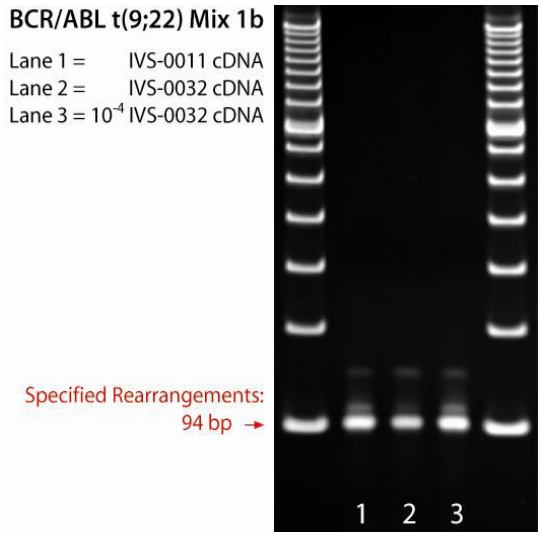

Figure 2. Amplified products were generated with *BCR/ABL* t(9;22) Master Mix 1b then run on a 2% agarose gel.

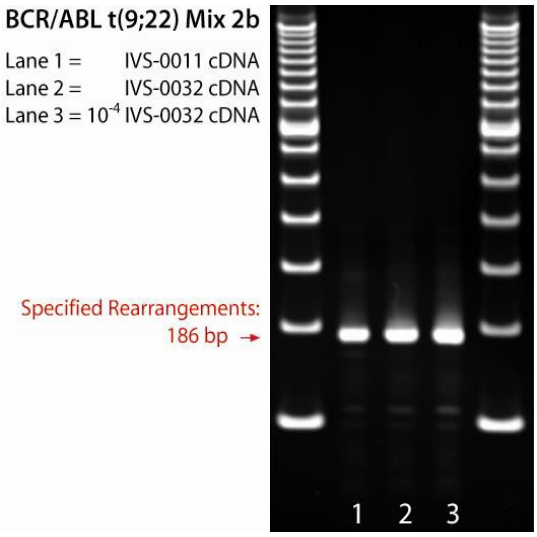

Figure 3. Amplified products were generated with *BCR/ABL* t(9;22) Master Mix 2b then run on a 2% agarose gel.

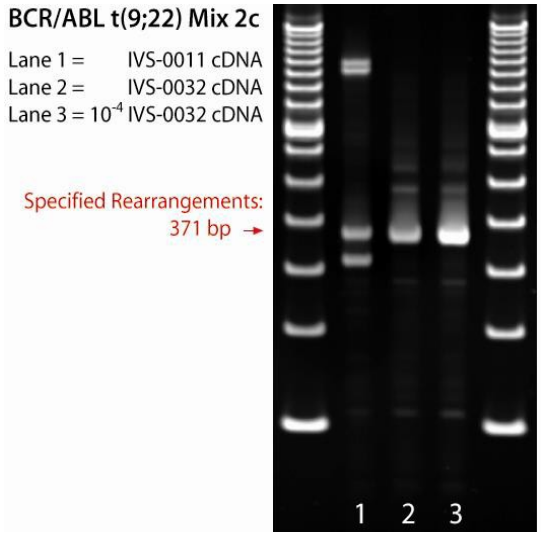

Figure 4. Amplified products were generated with *BCR/ABL* t(9;22) Master Mix 2c then run on a 2% agarose gel.

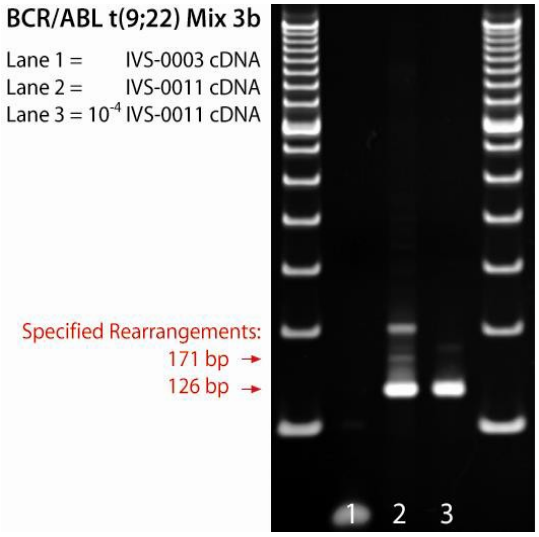

Figure 5. Amplified products were generated with *BCR/ABL* t(9;22) Master Mix 3b then run on a 2% agarose gel.

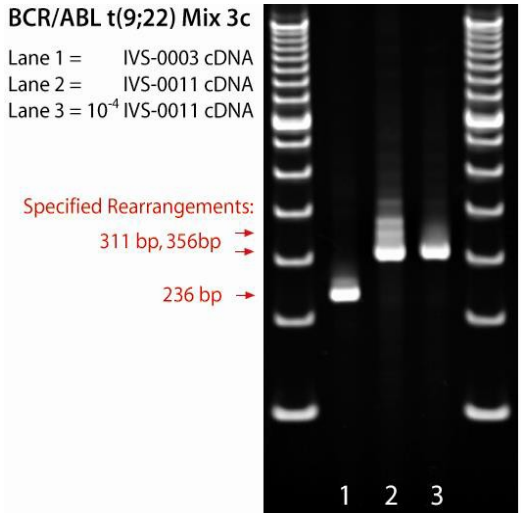

Figure 6. Amplified products were generated with *BCR/ABL* t(9;22) Master Mix 3c then run on a 2% agarose gel.

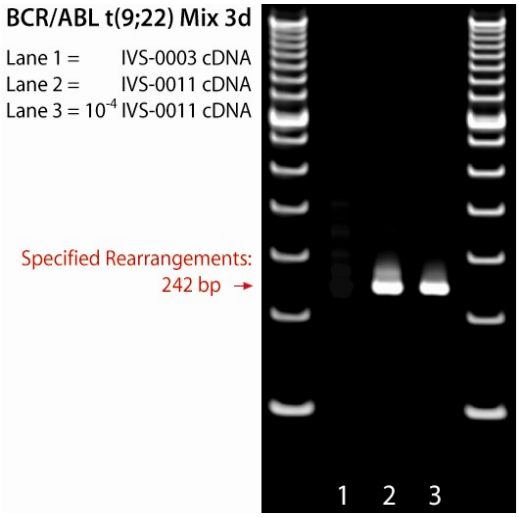

Figure 7. Amplified products were generated with *BCR/ABL* t(9;22) Master Mix 3d then run on a 2% agarose gel.

ABI Fluorescence Detection: The data shown in Figure 8 - Figure 13 were generated using the master mixes indicated then run on an ABI 3100 instrument. *BCR/ABL* t(9;22) Master Mixes 3a, 3b and 3c target the *BCR/ABL* p210- and p230-type translocations. IVS-0011 exhibits the p210 b3a2 product and amplifies with all 3 master mixes, whereas IVS-0003 exhibits the p210 b2a2 product and only amplifies with Master Mix 3c.

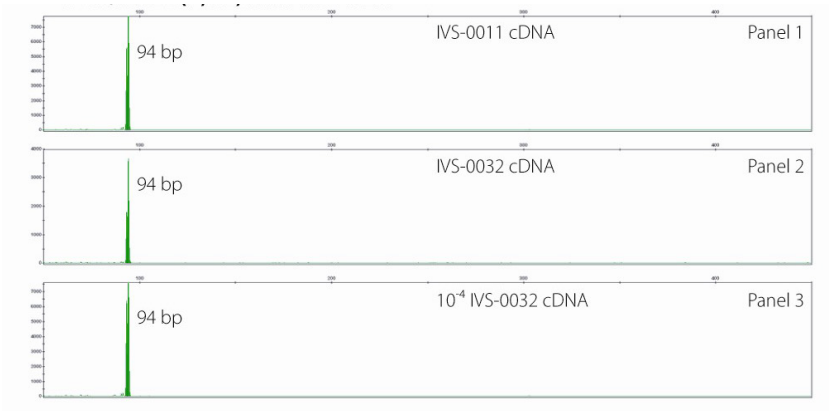

Figure 8. Amplified products were generated with *BCR/ABL* t(9;22) Master Mix 1b - HEX.

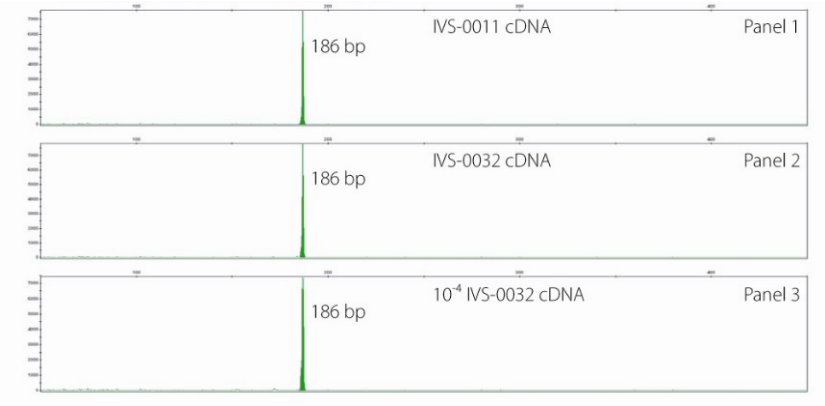

Figure 9. Amplified products were generated with *BCR/ABL* t(9;22) Master Mix 2b - HEX.

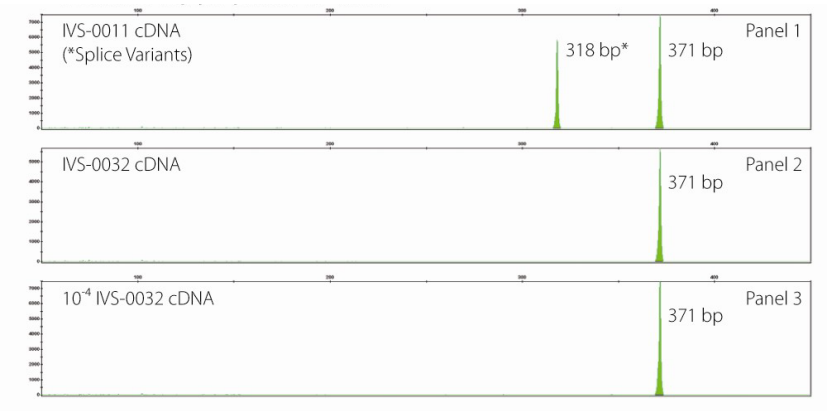

Figure 10. Amplified products were generated with *BCR/ABL* t(9;22) Master Mix 2c - HEX.

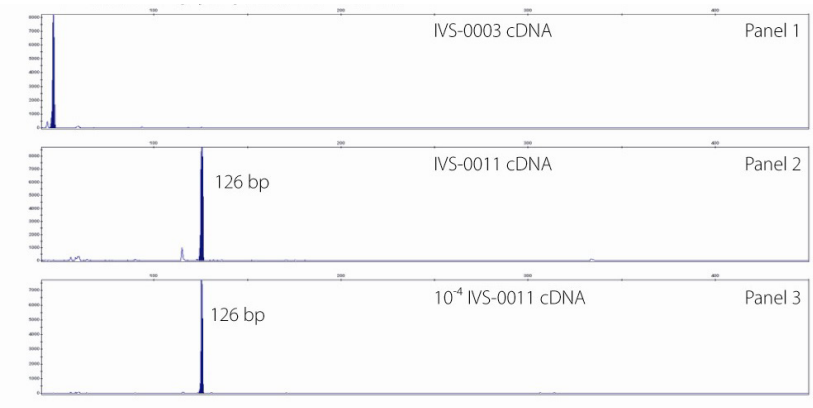

Figure 11. Amplified products were generated with *BCR/ABL* t(9;22) Master Mix 3b – 6FAM.

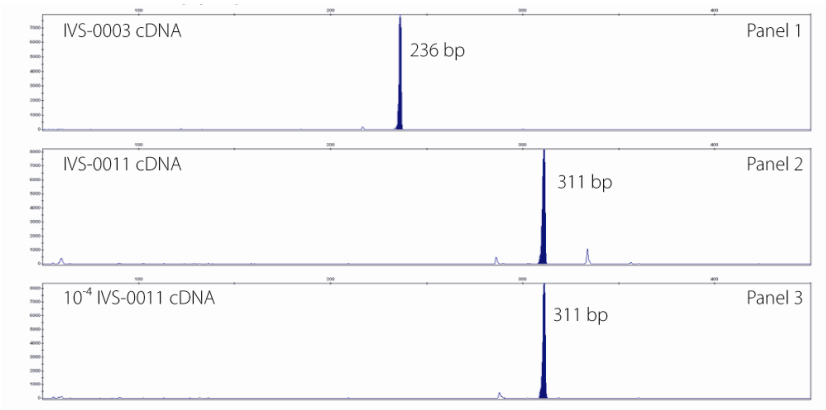

Figure 12. Amplified products were generated with *BCR/ABL* t(9;22) Master Mix 3c – 6FAM.

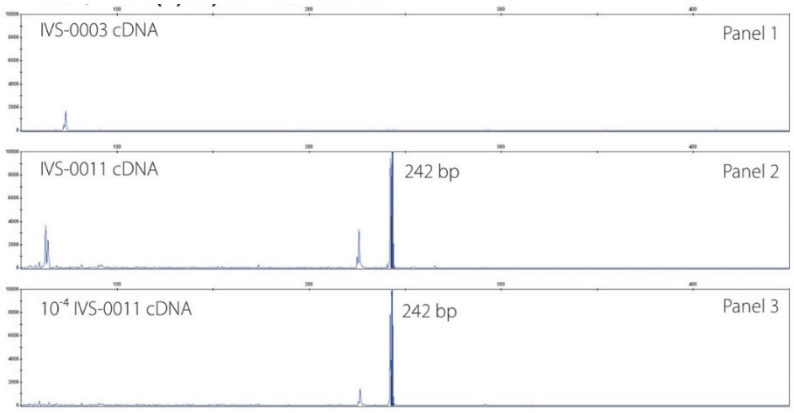

Figure 13. Amplified products were generated with *BCR/ABL* t(9;22) Master Mix 3d – 6FAM.

11. Technical and Customer Service

Thank you for purchasing our ***BCR/ABL* t(9;22) 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

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

12. References

- Deininger, MWN, Goldman, JM, and JV Melo. *Blood*. 96:3343-3356 (2000).
- Melo, JV. *Blood*. 88:2375 (1996).
- Miller, JE, Wilson, SS, Jaye, DL and Kronenberg, M. *Molecular Diagnosis*. 4: 101-117 (1999).
- Kurzrock, R. et al., *Ann. Intern. Med*. 138: 819-30 (2003).
- Radich, JP. et al., *Blood*. 85: 2632-2638 (1995).

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. *BCR/ABL* t(9;22) Translocation Assay: Single Page Guide

1st Round of Amplification Protocol:

15.1. In an containment hood or dead air box, thaw the 1st round master mixes: *BCR/ABL* t(9;22) Master Mixes 1a, 2a and 3a. Transfer volumes required for testing the samples and controls to separate microcentrifuge tubes (one tube for each master mix). Add DNA polymerase to these master mixes. Gently mix the tube contents. Aliquot 50 µL of the master mix/enzyme solutions into individual PCR wells or tubes.

15.2. Add 5 µL of sample or control cDNAs are added to the individual tubes or wells containing the respective master mix reactions. Amplify the reactions using the universal thermal cycler program.

15.3. Remove the amplification plate from the thermal cycler.

2nd Round of Amplification Protocol:

15.4. Dilute the 1st round PCR products from each reaction 1:100 (2 µL + 198 µL of molecular biology grade distilled water) and mix.

15.5. Thaw the 2nd round master mixes: BCR/ABL t(9;22) Master Mixes 1b, 2b, 2c, 3b, 3c, and 3d. Transfer the volumes required for testing the samples and controls to separate microcentrifuge tubes (one tube for each master mix).

15.6. Add DNA polymerase to these master mixes. Gently mix the tube contents.

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

15.8. Add 5 µL of the diluted amplification product from the 1st round reactions to the individual tubes or wells containing the respective master mix reactions:

- *BCR/ABL* t(9;22) Mix 1a product added into *BCR/ABL* t(9;22) Master Mix 1b
- *BCR/ABL* t(9;22) Mix 2a product added into *BCR/ABL* t(9;22) Master Mixes 2b and 2c
- *BCR/ABL* t(9;22) Mix 3a product added into *BCR/ABL* t(9;22) Master Mixes 3b, 3c and 3d

15.9. Amplify the reactions using the same PCR program, above.

Gel Detection – Agarose TBE Gels

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

15.11. Individually mix 20 µL from each amplification reaction with 4 µL of 6X gel loading buffer. Add 20 µL of this mixture to separate wells of the gel, flanked by DNA size standards.

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

15.13. Photograph the gel and interpret the data.

ABI Fluorescence Detection with ABI 310 & 3100 instruments

15.14. Add 1 µL of reaction products from the BCR/ABL t(9;22) Master Mixes 1b, 2b, 2c, 3b, 3c, and 3d in separate tubes; then add 10 µL of HI-Deionized Formamide containing ROX size standards to each tube. Mix well.

15.15. Heat PCR products to 95ºC for 2 minutes then snap chill on ice for 5 minutes.

15.16. Prepare a sample sheet and injection list for the samples.

15.17. Data are automatically displayed as size and color specific peaks. Review profile and controls, report results.

ABI Fluorescence Detection with ABI 3500 instruments

Note: Due to instrument to instrument variation in the performance of the ABI 3500 platform, the amount of formamide, sample and size standard listed in the protocol is intended to be a starting point. The protocol may need to be optimized for specific ABI 3500 Platforms.

15.18. In a new microcentrifuge tube, mix an appropriate amount (9.5 µL per reaction) of Hi-Di Formamide with LIZ Size Standards. Vortex well.

15.19. In a new 96-well PCR plate, add 9.5 µL of Hi-Di Formamide with LIZ size standards to individual wells for each reaction.

15.20. Transfer 0.5 µL of each reaction to the wells containing Hi-Di Formamide with LIZ size standards. Add only one sample per well. Pipette up and down to mix.

15.21. Cap or cover the PCR plate.

15.22. Heat denature the samples at 95ºC for 3 minutes, then snap chill on ice for 5 minutes.

15.23. Prepare a sample sheet and injection list for the samples.

15.24. Run the samples on an ABI 3500 capillary electrophoresis instrument according to its user manual.

15.25. Review profile and controls, report results.