GeneXpert
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Xpert® BCR-ABL Ultra p190

REF **RBCRABLP190-10**

Instructions for Use
For Use with GeneXpert Dx System
Research Use Only (RUO)

RUO

Cepheid®

For Research Use Only. Not for use in Diagnostic procedures.

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Trademark, Patents and Copyright Statements

See Section 24, Revision History for a description of changes.

Xpert® BCR-ABL Ultra p190

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1 Proprietary Name

Xpert® BCR-ABL Ultra p190

2 Common or Usual Name

Xpert BCR-ABL Ultra p190

3 Product Description

The Xpert BCR-ABL Ultra p190 assay, performed on the GeneXpert® Dx System, is a real-time RT-PCR (reverse transcription-polymerase chain reaction) assay for the quantitative detection of the BCR-ABL1 p190 chromosomal translocation mRNA transcript (type e1a2) and the ABL1 endogenous control mRNA transcript in peripheral blood samples.

4 Summary and Explanation

The Philadelphia (Ph) chromosome is a shortened chromosome that results from the transposition of the 3' part of the ABL gene on chromosome 9 to the 5' part of the BCR gene on chromosome 22. The breakpoint on the ABL gene is fairly constant occurring at the 5' end of exon a2 whereas the breakpoint of the BCR gene is variable but is mainly clustered in 3 different regions (breakpoint cluster regions or bcr). Depending on the breakpoint on chromosome 22, different size segments are joined with the 3' sequences of the ABL gene. There are major (M-bcr), minor (m-bcr) and micro-breakpoints, each of which result in different size mRNA fusion transcripts.[1]

The M-bcr results in the e13a2(b2a2) and e14a2(b3a2) fusion transcripts which are translated into BCR-ABL p210 proteins. The m-bcr results in the e1a2 fusion transcript and is translated into the BCR-ABL p190 protein. The e19a2 micro-breakpoint is translated into the BCR-ABL p230 protein.[1,2]

The Ph chromosome is observed in greater than 95% of individuals with chronic myeloid leukemia (CML) and up to 20-30% of adults with acute lymphoblastic leukemia (ALL), 5% of children with ALL and in 1-2% of individuals with acute myeloid leukemia (AML).[1,3]

In CML, the BCR-ABL p210 is present in greater than 95% of individuals and is also found in approximately 30% of Ph-positive (Ph+) ALL individuals. In the remaining individuals with Ph+ ALL and in rare cases of CML (1-3%), the BCR-ABL p190 is present.

In CML, the BCR-ABL p210 and p190 can co-exist. Both the p210 and p190 fusion proteins demonstrate increased tyrosine phosphokinase activity compared to the normal p145 c-abl protein.[1,2,4,5]

In Ph+ ALL individuals, the p190 form is detected in approximately 80% of Ph+ childhood ALL and 20-40% of Ph+ adult ALL.[1]

5 Principle of the Procedure

The Xpert BCR-ABL Ultra p190 assay is an automated assay for quantifying the amount of BCR-ABL1 p190 transcript as a ratio of BCR-ABL1 p190/ABL1. The assay is performed on Cepheid GeneXpert Dx System, which automates and integrates sample purification, nucleic acid amplification, and target sequence detection in simple or complex samples using real-time RT-PCR and nested PCR assays. The system consists of an instrument, computer, and pre-loaded software for running assays and viewing the results. The system requires the use of single-use, disposable GeneXpert cartridges that hold the RT-PCR and nested PCR reagents and host the RT-PCR and nested PCR processes. For a full description of the system, refer to the appropriate *GeneXpert Dx System Operator Manual*.

The Xpert BCR-ABL Ultra p190 assay includes reagents to detect BCR-ABL1 p190 fusion genes resulting from a minor breakpoint, translocation e1a2, and the ABL1 transcript as an endogenous control in peripheral blood samples. The amount of BCR-ABL1 p190 transcript is quantified as the percent ratio of BCR-ABL1 p190/ABL1. There are two controls included in the Xpert BCR-ABL Ultra p190 assay – the Endogenous Control (ABL1) and a Probe Check Control (PCC). The ABL1 endogenous control normalizes the BCR-ABL1 p190 target and ensures that sufficient sample is used in the assay. The PCC verifies reagent rehydration, PCR tube filling, and that all reaction components, including probes and dyes, are present and functional in the cartridge.

6 Reagents and Instruments

6.1 Material Provided

The Xpert BCR-ABL Ultra p190 kit (RBCRABLP190-10) contains sufficient reagents to process 10 test samples or quality control samples. The kit contains the following:

Xpert BCR-ABL Ultra Reagents	**10 of each per kit**
• Proteinase K (PK)	10 x 130 µL per vial
• Lysis Reagent (LY) (Guanidinium Chloride)	10 x 5.3 mL per vial

• Wash Reagent (1)
  • Ethanol
  • Guanidinium thiocyanate
	10 x 2.9 mL per ampoule

Xpert BCR-ABL Ultra p190 Cartridges with Integrated Reaction Tubes	**10 per kit**
• Bead 1, 2, 3 and 4 (freeze-dried)	1 of each per cartridge
• Rinse Reagent	2.0 mL per cartridge
• Elution Reagent	2.5 mL per cartridge
CD	**1 per kit**
• Assay Definition File (ADF)	
• Instruction to import ADF into GeneXpert Dx software	
• Instructions for Use (Package Insert)	

Note Safety Data Sheets (SDS) are available at www.cepheid.com or www.cepheidinternational.com under the **SUPPORT** tab.

Note The bovine serum albumin (BSA) in the beads within this product was produced and manufactured exclusively from bovine plasma sourced in the United States. No ruminant protein or other animal protein was fed to the animals; the animals passed ante- and post-mortem testing. During processing, there was no mixing of the material with other animal materials.

6.2 Materials Required but Not Provided

- GeneXpert Dx System (catalog number varies by configuration): GeneXpert instrument, computer, barcode scanner, and operator manual.
- For GeneXpert System: GeneXpert Dx software version 6.2 or higher.
- Printer: If a printer is required, contact Cepheid Technical Support to arrange for the purchase of a recommended printer.
- Vortex mixer
- Microcentrifuge (1000 x g minimum)
- Pipettes and aerosol filter pipette tips
- 50 mL conical tubes
- Reagent grade absolute ethanol

6.3 Materials Available but Not Required

Xpert BCR-ABL Ultra p190 External Controls, INTROL® BCR-ABL1 p190 Control Panel, Catalog number C183, are quality controls from Maine Molecular Quality Controls, Inc.

7 Storage and Handling

- Store the Xpert BCR-ABL Ultra p190 kit contents at 2°C to 8°C until the expiration date provided on the label.
- Do not open the cartridge lid until you are ready to perform the assay.
- Do not use cartridges that have passed the expiration date.
- Do not use a cartridge that has leaked.
- The Wash Reagent is a clear, colorless liquid. Do not use the Wash Reagent if it has become cloudy or discolored.
- Twenty (20) minutes before starting the procedure, remove the blood sample, cartridge and sample preparation reagents from storage to allow them to come to room temperature (20°C to 30°C).

8 Warnings and Precautions

8.1 General

- For Research Use Only. Not for use in diagnostic procedures.
- Treat all biological samples, including used cartridges and reagents, as if capable of transmitting infectious agents. Because it is often impossible to know which might be infectious, all biological samples should be treated with standard precautions. Guidelines for sample handling are available from U.S. Centers for Disease Control and Prevention[6] and Clinical and Laboratory Standards Institute.[7]
- Follow safety procedures set by your institution for working with chemicals and handling biological samples.
- The assay function has been established with blood collected in EDTA tubes only. The assay function has not been evaluated with other sample types.
- Reliable results are dependent on adequate sample collection, transport, storage and processing. Incorrect assay results may occur from improper sample collection, handling or storage, technical error, sample mix-up or because the target transcript in the sample is below the limit of detection of the assay. Careful compliance with the Package Insert instructions and the *GeneXpert Dx System Operator Manual* are necessary to avoid erroneous results.
- Performing the Xpert BCR-ABL Ultra p190 assay outside the recommended kit or sample storage temperature ranges and time may produce erroneous or invalid results.
- Biological samples, transfer devices, and used cartridges should be considered capable of transmitting infectious agents requiring standard precautions. Follow your institution's environmental waste procedures for proper disposal of used cartridges and unused reagents. These materials may exhibit characteristics of chemical hazardous waste requiring specific national or regional disposal procedures. If national or regional regulations do not provide clear direction on proper disposal, biological samples and used cartridges should be disposed per WHO [World Health Organization] medical waste handling and disposal guidelines.[8]

8.2 Sample

- Maintain proper storage conditions during sample transport to ensure the integrity of the sample (see Section 10, Sample Collection, Transport and Storage). Sample stability under shipping conditions other than those recommended has not been evaluated.
- Do not freeze whole blood samples.
- Proper sample collection, storage, and transport are essential for correct results.

8.3 Assay/Reagent

- Do not substitute Xpert BCR-ABL Ultra reagents with other reagents.
- Do not open the Xpert BCR-ABL Ultra p190 cartridge lid except when adding sample and Wash Reagent.
- Do not use a cartridge that has been dropped after removing it from the packaging.
- Do not shake the cartridge. Shaking or dropping the cartridge after opening the cartridge lid may yield invalid results.
- Do not place the sample ID label on the cartridge lid or on the barcode label of the cartridge.
- Do not use a cartridge with a damaged barcode label.
- Do not use a cartridge that has a damaged reaction tube.
- It is recommended that the Xpert BCR-ABL Ultra p190 cartridges be at room temperature (20°C to 30°C) when used for testing.
- Each single-use Xpert BCR-ABL Ultra p190 cartridge is used to process one assay.
- Do not reuse processed cartridges.
- Do not reuse pipette tips.
- Do not use a cartridge if it appears wet or if the lid seal appears to have been broken.
- Do not use the Xpert BCR-ABL Ultra p190 cartridge if a reagent is added to the wrong opening.
- Do not open Xpert BCR-ABL Ultra p190 cartridges after the assay is completed.
- Dedicate a set of pipettes and reagents exclusively to sample preparation.
- Wear clean lab coats and gloves.
- Change gloves between the handling of each sample.
- In the event of a spill of samples or controls, wear gloves and absorb the spill with paper towels. Then, thoroughly clean the contaminated area with a 1:10 dilution of freshly prepared household chlorine bleach. Final active chlorine concentration should be 0.5% regardless of the household bleach concentration in your country. Allow a minimum of two minutes of contact time.
- Ensure the work area is dry before using 70% denatured ethanol to remove bleach residue. Allow surface to dry completely before proceeding. Or, follow your institution's standard procedures for a contamination or spill event. For equipment, follow the manufacturer's recommendations for decontamination of equipment.

9 Chemical Hazards

Note The information below applies to the entire product containing Proteinase K, Lysis, Wash, and Rinse Reagents.

- CLP/GHS Hazard Pictogram: 
- Signal Word: DANGER
- **UN GHS Hazard Statements**
  - Highly flammable liquid and vapour.
  - Causes skin irritation.
  - Causes serious eye irritation.
  - May cause drowsiness or dizziness.
  - Suspected of causing genetic defects.
- **UN GHS Precautionary Statements**
  - **Prevention**
    - Obtain special instructions before use.
    - Do not handle until all safety precautions have been read and understood.
    - Keep away from heat, sparks, open flames and/or hot surfaces. No smoking.
    - Keep container tightly closed.

- Avoid breathing mist/vapours/spray.
- Wash thoroughly after handling.
- Use only outdoors or in a well-ventilated area.
- Wear protective gloves/protective clothing/eye protection/face protection.
- Use personal protective equipment as required.

- **Response**
  - In case of fire: Use appropriate media for extinction.
  - IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
  - Call a POISON CENTER or doctor/physician if you feel unwell.
  - IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.
  - Specific treatment, see supplemental first aid information.
  - Take off contaminated clothing and wash before reuse.
  - If skin irritation occurs: Get medical advice/attention.
  - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
  - If eye irritation persists: Get medical advice/attention.
  - IF exposed or concerned: Get medical advice/attention.
- **Storage/Disposal**
  - Keep cool.
  - Store in a well-ventilated place.
  - Keep container tightly closed.
  - Store locked up.
  - Dispose of content and/or container in accordance with local, regional, national, and/or international regulations.

10 Sample Collection, Transport and Storage

- Peripheral blood samples should be collected in EDTA tubes following your institution's guidelines. Plasma should not be separated from cells.
- Samples should be stored at 2°C to 8°C for no longer than 3 days (72 hours) prior to testing.
- Proper sample collection, storage, and transport are critical to the assay function. Sample stability under shipping and storage conditions other than those listed below have not been evaluated with the Xpert BCR-ABL Ultra p190 assay.

11 Procedure

11.1 Before You Start

Twenty (20) minutes before starting the procedure, remove the blood sample, sample preparation reagents, and cartridges from refrigerated storage to allow them to come to room temperature. Briefly spin down the Proteinase K (PK) in a microcentrifuge.

Important **Start the assay within 1 hour of adding the Sample Reagent-treated sample to the cartridge.**

Important **Remove the cartridge from the cardboard packaging before preparing the sample. (See Section 11.3, Preparing the Cartridge).**

11.2 Preparing the Sample

11.2.1 Preparing a Sample with Unknown White Blood Cell (WBC) Count or with No Greater than 30 Million WBC/mL

1. To the bottom of a new 50 mL conical tube, add 100 µL of PK (Proteinase K).
2. Ensure blood sample is well-mixed by inverting the blood collection tube 8 times immediately before pipetting. See manufacturer's instructions for the EDTA blood collection tube.
3. To the tube already containing Proteinase K, add 4 mL of blood sample.
4. Mix the sample with a vortex mixer at maximum setting continuously for 3 seconds.
5. Incubate at room temperature for 1 minute.
6. To the same tube, add 2.5 mL of Lysis Reagent (LY).

Note Retain the remaining lysis reagent to use again in Step 13.

7. Mix the sample with a vortex mixer at maximum setting continuously for 10 seconds.
8. Incubate at room temperature for 5 minutes.
9. Mix the sample with a vortex mixer at maximum setting continuously for 10 seconds.
10. Incubate at room temperature for 5 minutes.
11. Mix the sample by tapping the bottom of the tube 10 times.
12. Transfer 1 mL of the prepared lysate into a new 50 mL conical tube.

Note Remaining lysate can be stored at 2–8 ºC for up to 48 hours or stored at -20 ºC or lower for up to 3 months.

13. To the new conical tube containing lysate, add 1.5 mL of retained Lysis Reagent (LY) from Step 6.
14. Mix the sample with a vortex mixer at maximum setting continuously for 10 seconds.
15. Incubate at room temperature for 10 minutes.
16. To the same conical tube, add 2 mL of reagent grade absolute ethanol (provided by user).
17. Mix the sample with a vortex mixer at maximum setting continuously for 10 seconds. Set aside.
18. Discard any remaining PK or LY reagents.

11.2.2 Preparing a Sample with WBC Count Greater than 30 Million cells/mL

1. To the bottom of a new 50 mL conical tube, add 100 µL of PK (Proteinase K).
2. Ensure blood specimen is well-mixed by inverting the blood collection tube 8 times immediately before pipetting. See manufacturer's instructions for the EDTA blood collection tube.
3. To the tube already containing Proteinase K, add 50 µL of blood specimen.
4. Mix the sample with a vortex mixer at maximum setting continuously for 3 seconds.
5. Incubate at room temperature for 1 minute.
6. To the same tube, add 2.5 mL of Lysis Reagent (LY).
7. Mix the sample with a vortex mixer at maximum setting continuously for 10 seconds.
8. Incubate at room temperature for 5 minutes.
9. Mix the sample with a vortex mixer at maximum setting continuously for 10 seconds.
10. Incubate at room temperature for 5 minutes.
11. To the same conical tube, add 2 mL of reagent grade absolute ethanol (provided by user).
12. Mix the sample with a vortex mixer at maximum setting continuously for 10 seconds. Set aside.
13. Discard any remaining PK or LY reagents.

11.3 Preparing the Cartridge

To add the sample to the Xpert BCR-ABL Ultra p190 cartridge:

1. Remove the cartridge from the cardboard packaging.
2. Inspect the cartridge for damage. If damaged, do not use it.
3. Open the cartridge by lifting the cartridge lid and transfer the entire contents of the Wash Reagent (1) ampoule to the Wash Reagent Chamber (with small opening). See Figure 1.
4. Pipette the entire contents of the prepared sample into the Sample Chamber (large opening). See Figure 1.

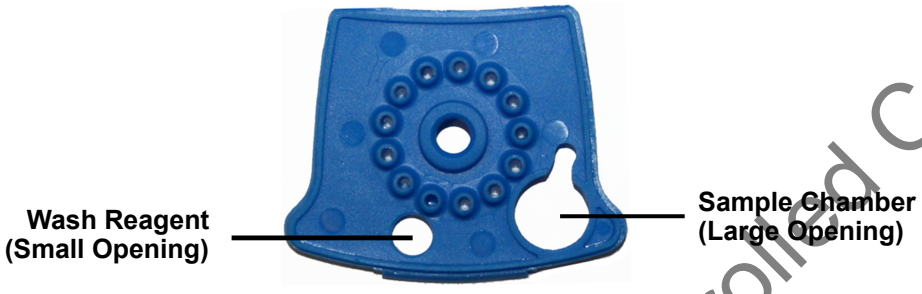

Figure 1. Xpert BCR-ABL Ultra p190 Cartridge (Top View)

5. Close the cartridge lid. Ensure the lid snaps firmly into place. Initiate assay (see Section 11.4, Starting the Assay).

11.4 Starting the Assay

Important **Start the assay within 1 hour of adding the sample to the cartridge.**

Important **Before you start the assay, make sure that the system is running GeneXpert Dx software version 6.2 or higher and that the Xpert BCR-ABL Ultra p190 assay definition file is imported into the software. This section lists the default steps to operate the GeneXpert Dx System.**

Note The steps you follow may be different if the system administrator has changed the default workflow of the system.

1. Turn on the GeneXpert system by first turning on the GeneXpert Dx instrument and then turning on the computer. The GeneXpert Dx software will launch automatically or may require double-clicking the GeneXpert Dx software shortcut icon on the Windows® desktop.
2. Log on to the GeneXpert software using your user name and password.
3. In the GeneXpert window, click **Create Test** (GeneXpert Dx). The Create Test window opens.
4. Scan or type in the Sample ID. If typing the Sample ID, make sure the Sample ID is typed correctly. The Sample ID is shown on the left side of the View Results window and is associated with the test results.
5. Scan the barcode on the Xpert BCR-ABL Ultra p190 cartridge. Using the barcode information, the software automatically fills the boxes for the following fields: Reagent Lot ID, Cartridge SN, and Expiration Date.

Note If the barcode on the Xpert BCR-ABL Ultra p190 cartridge does not scan, then repeat the assay with a new cartridge.

6. Click **Start Test** (GeneXpert Dx). Type your password in the dialog box that appears.
7. Open the instrument module door with the blinking green light and load the cartridge.
8. Close the door. The assay starts and the green light stops blinking. When the assay is finished, the light turns off.
9. Wait until the system releases the door lock before opening the module door and removing the cartridge.
10. Dispose of used cartridges in the appropriate sample waste container according to your institution's standard practices.

Note Time to result is less than 2.5 hours (approximately 30 minutes offboard sample preparation and 1 hour 45 minutes assay run time).

12 Viewing and Printing Results

This section lists the basic steps for viewing and printing results. For more detailed instructions on how to view and print the results, see the *GeneXpert Dx System Operator Manual*.

1. Click the **View Results** icon to view results.
2. Upon completion of the assay, click the **Report** button of the View Results screen to view and/or generate a PDF report file.

13 Quality Control

Each cartridge includes an ABL 1 Endogenous Control and a Probe Check Control (PCC).

ABL 1 Endogenous Control — The ABL 1 Endogenous Control verifies that sufficient sample is used with the assay. Additionally, this control detects sample-associated inhibition of the real-time PCR assay. The ABL 1 passes if it meets the assigned acceptance criteria.

Probe Check Control (PCC) — Before the start of the PCR reaction, the GeneXpert system measures the fluorescence signal from the probes to monitor bead rehydration, reaction tube filling, and if all reaction components are functional in the cartridge. The PCC passes if it meets the assigned acceptance criteria.

14 Interpretation of Results

The results are interpreted automatically by the GeneXpert system from measured fluorescent signals and embedded calculation algorithms and are clearly shown in the View Results window. The possible results and interpretations are shown in Table 1.

Table 1. Xpert BCR-ABL Ultra p190 Possible Results and Interpretation

Result	Interpretation

BCR-ABL p190 DETECTED
See Figure 2, Figure 3, Figure 4
	BCR-ABL p190 transcript was detected.
	● BCR-ABL p190 DETECTED – BCR-ABL p190 transcript was detected and has a cycle threshold (Ct) within the valid range and endpoint above the threshold setting.
	● Possible positive results:
	  ● BCR-ABL p190 DETECTED [#.##%]; Figure 2.
	  ● BCR-ABL p190 DETECTED [Above upper LoQ]; Figure 3.
	  ● BCR-ABL p190 DETECTED [Below LoD; <#.###%]; Figure 4.
	● ABL PASS – ABL transcript was detected and has a cycle threshold (Ct) within the valid range and endpoint above the threshold setting.
	● Probe Check PASS – all probe check results passed.

BCR-ABL p190 NOT DETECTED See Figure 5	BCR-ABL p190 transcript was not detected. ● BCR-ABL p190 NOT DETECTED [Sufficient ABL transcript] – BCR-ABL p190 transcript was not detected and has a cycle threshold (Ct) above the valid cycle threshold. ● ABL PASS – ABL transcript was detected and has a cycle threshold (Ct) within the valid range and endpoint above the threshold setting. ● Probe Check PASS – all probe check results passed.
INVALID See Figure 6, Figure 7, Figure 8	BCR-ABL p190 transcript level cannot be determined. ● INVALID – BCR-ABL p190 transcript level cannot be determined due to sample containing excess BCR-ABL p190 and/or ABL transcripts. See Section 17, Troubleshooting Guide, for additional instructions for retesting the sample. ● ABL FAIL – ABL cycle threshold (Ct) was not within the valid range or the endpoint was below the threshold setting (Figure 7). See Section 17, Troubleshooting Guide, for additional instructions for retesting the sample. ● Probe Check – PASS; all probe check results passed.
ERROR See Figure 9	BCR-ABL p190 transcript level cannot be determined. See Section 17, Troubleshooting Guide, for additional instructions for retesting the sample. ● BCR-ABL p190 – NO RESULT ● ABL – NO RESULT ● Probe Check FAIL – All or one of the probe check results failed. ● Probe Check PASS or NA (not applicable) and Pressure Abort. *If the probe check passed or shows N/A, the error was caused by the maximum pressure limit exceeding the acceptable range or by a system component failure.
NO RESULT	BCR-ABL p190 transcript level cannot be determined. Insufficient data were collected to produce a test result. For example, this can occur if the operator stopped an assay that was in progress. See Section 17, Troubleshooting Guide, for additional instructions for retesting the sample. ● BCR-ABL p190 NO RESULT ● ABL NO RESULT ● Probe Check NA (not applicable)

15 Quantitative Results

Xpert BCR-ABL Ultra p190 quantitative outputs are provided as a percent ratio of BCR-ABL1 p190/ABL1. Kits are assigned lot-specific Efficiency (EΔCt) and Scaling Factor (SF) values that tie the quantitation of BCR-ABL1 p190 (e1a2) and ABL1 transcripts to copy numbers of synthetic BCR-ABL p190 and ABL1 RNA in vitro transcribed RNA (IVT-RNA) primary standards.

Note GeneXpert systems calculate results automatically using Efficiency and Scaling Factor values embedded within each cartridge barcode. Efficiency and Scaling Factor values are also available upon request.

Table 2. Examples of Xpert BCR-ABL Ultra p190 Assay Results

Test	BCR-ABL p190		ABL		Xpert BCR-ABL Ultra p190 Assay Results	Notes
	Ct	Result	Ct	Result		
1	7.1	INVALID	7.3	FAIL	INVALID [Too high BCR-ABL p190 and ABL transcripts]	Calculated % value: 203.17%
2	8.1	INVALID	7.9	FAIL	INVALID [Too high ABL transcript]	Calculated % value: 152.46%
3	7.9	INVALID	8.1	PASS	INVALID [Too high BCR-ABL p190 transcript]	Calculated % value: 203.17%
4	25.0	INVALID	18.2	FAIL	INVALID [Insufficient ABL transcript]	NA
5	0	INVALID	0	FAIL	INVALID [No ABL transcript]	NA
6	12.3	POS	11.2	PASS	BCR-ABL p190 DETECTED [Above upper LoQ]	Calculated % value: 79.91%
7	22.5	POS	11.8	PASS	BCR-ABL p190 DETECTED [0.081%]	Calculated % value: 0.081%
8	27.8	POS	11.6	PASS	BCR-ABL p190 DETECTED [Below LoD; <0.0060%]	Calculated % value: 0.0016%
9	0	NEG	12.1	PASS	BCR-ABL p190 NOT DETECTED [Sufficient ABL transcript]	0%
10	0	NO RESULT	0	NO RESULT	ERROR	For example, Error 5017 [ABL] probe check failed

15.1 BCR-ABL p190 DETECTED [#.##]%

BCR-ABL p190 has been detected at a level of #.##%.

For a "BCR-ABL p190 DETECTED [#.##%]" result, BCR-ABL p190 is detectable with BCR-ABL p190 Ct greater than or equal to "8" and less than or equal to the cut-off of "32" and ABL Ct greater than or equal to "8" and less than or equal to "18". The GeneXpert software calculates the % using the following equation where the Delta Ct (ΔCt) value is obtained from ABL Ct minus BCR-ABL p190 Ct:

$\% = E_{\Delta Ct}^{(\Delta Ct)}$ x 100 x Scaling Factor *(SF)*

Note The Scaling Factor (*SF*) is a lot-specific parameter that is embedded within the assay cartridge barcode. The value of this factor and the lot-specific assay Efficiency ($E_{\Delta Ct}$) are determined in quality control testing of each assay lot using secondary standards calibrated to the copy numbers of the synthetic BCR-ABL p190 and ABL1 RNA *in vitro* transcribed RNA (IVT-RNA) calibrators for quantitation of BCR-ABL p190 transcript. The $E_{\Delta Ct}$ is set for 2.05 and *SF* value is set for 1.76 for use in the example shown here.

Example: Lot-specific $E_{\Delta Ct}$ = 2.05; *SF* = 1.76
Assay's ABL Ct = 11.4; BCR-ABL p190 Ct = 15.6 ; ΔCt = -4.2
$\% = 2.05^{(-4.2)}$ x 100 x 1.76 = 8.63%

Result: **BCR-ABL p190 DETECTED [8.63%]**. See Figure 2.

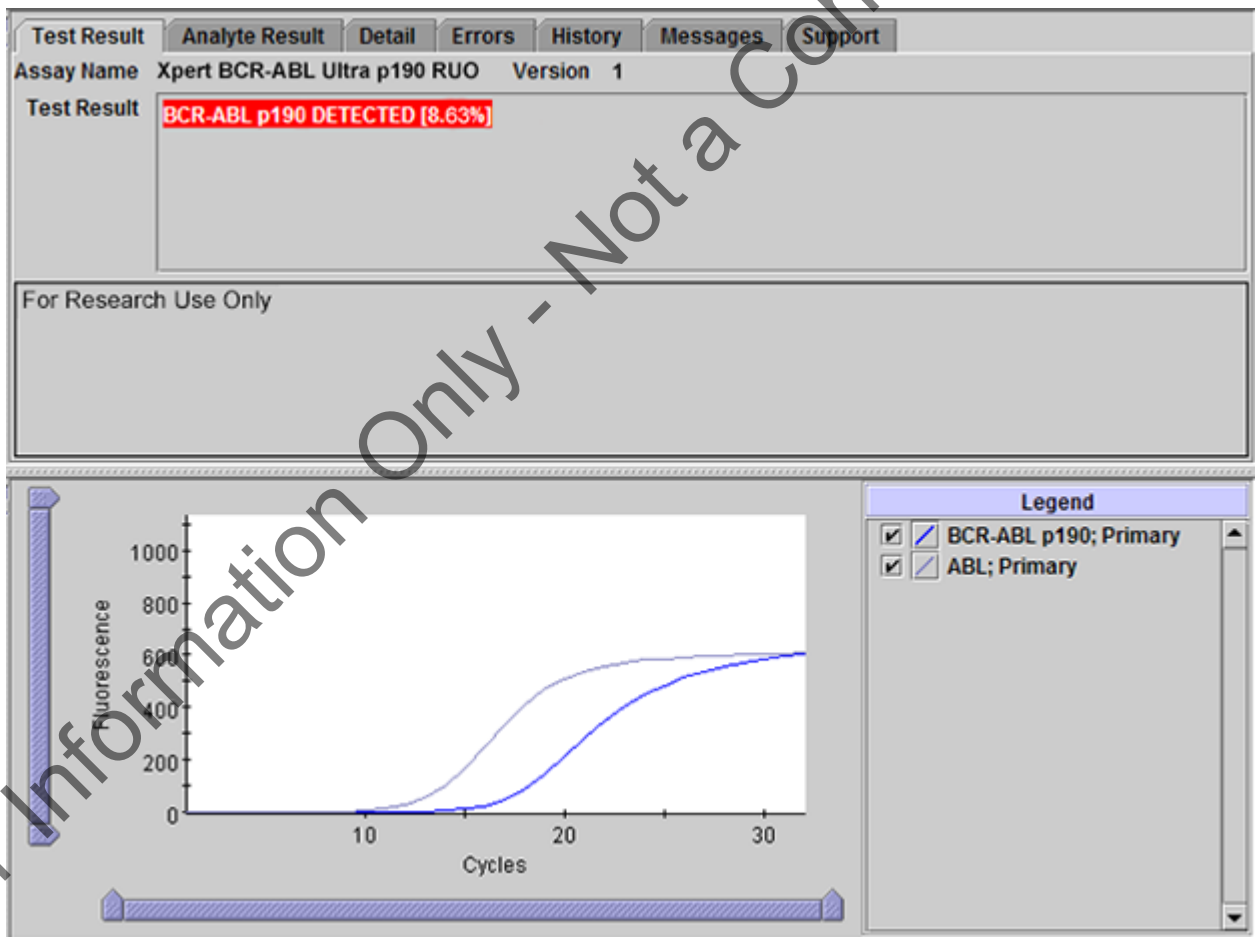

Figure 2. GeneXpert Dx View Results Window: BCR-ABL p190 DETECTED [8.63%]

15.2 BCR-ABL p190 DETECTED [Above upper LoQ]

BCR-ABL p190 has been detected at a level > 25%.

For a "BCR-ABL p190 DETECTED [Above upper LoQ]" result, BCR-ABL p190 is detectable with BCR-ABL p190 Ct greater than or equal to "8" and less than or equal to the cut-off of "32" and ABL Ct greater than or equal to "8" and less than or equal to "18". The GeneXpert software calculates the % using the following equation where the Delta Ct (ΔCt) value is obtained from ABL Ct minus BCR-ABL p190 Ct:

$\% = E_{\Delta Ct}^{(\Delta Ct)}$ x 100 x Scaling Factor *(SF)*

Note The Scaling Factor (*SF*) is a lot-specific parameter that is embedded within the assay cartridge barcode. The value of this factor and the lot-specific assay Efficiency ($E_{\Delta Ct}$) are determined in quality control testing of each assay lot using secondary standards calibrated to the copy numbers of the synthetic BCR-ABL p190 and ABL1 RNA *in vitro* transcribed RNA (IVT-RNA) calibrators for quantitation of BCR-ABL p190 transcript. The $E_{\Delta Ct}$ is set for 2.05 and *SF* value is set for 1.76 for use in the example shown here.

Example: Lot-specific $E_{\Delta Ct}$ = 2.05; *SF* = 1.76
Assay's ABL Ct = 17.2; BCR-ABL p190 Ct = 18.7; ΔCt = -1.6
$\% = 2.05^{(-1.6)}$ x 100 x 1.76 = 56.6% is greater than the defined assay upper LoQ at 25%

Result: **BCR-ABL p190 DETECTED [Above upper LoQ]**. See Figure 3.

Figure 3. GeneXpert Dx View Results Window: BCR-ABL p190 DETECTED [Above upper LoQ]

15.3 BCR-ABL p190 DETECTED [Below LoD; <0.0060%]

BCR-ABL p190 has been detected at a level < 0.0060%.

For a "BCR-ABL p190 DETECTED [Below LoD; <0.0060%]" result, BCR-ABL p190 is detectable with BCR-ABL p190 Ct greater than or equal to "8" and less than or equal to the cut-off of "32" and ABL Ct greater than or equal to "8" and less than or equal to "18". The GeneXpert software calculates the % using the following equation where the Delta Ct (ΔCt) value is obtained from ABL Ct minus BCR-ABL p190 Ct:

$\% = E_{\Delta Ct}^{(\Delta Ct)} \times 100 \times$ Scaling Factor *(SF)*

Note The Scaling Factor (*SF*) is a lot-specific parameter that is embedded within the assay cartridge barcode. The value of this factor and the lot-specific assay Efficiency ($E_{\Delta Ct}$) are determined in quality control testing of each assay lot using secondary standards calibrated to the copy numbers of the synthetic BCR-ABL p190 and ABL1 RNA *in vitro* transcribed RNA (IVT-RNA) calibrators for quantitation of BCR-ABL p190 transcript. The $E_{\Delta Ct}$ is set for 2.05 and *SF* value is set for 1.76 for use in the example shown here.

Example: Lot-specific $E_{\Delta Ct}$ = 2.05; *SF* = 1.76
Assay's ABL Ct = 10.1; BCR-ABL p190 Ct = 24.8; ΔCt = -14.8
$\% = 2.05^{(-14.8)} \times 100 \times 1.76 = 0.0044\%$ is less than the defined assay LoD at 0.0060%

Result: **BCR-ABL p190 DETECTED [Below LoD; <0.0060%]**. See Figure 4.

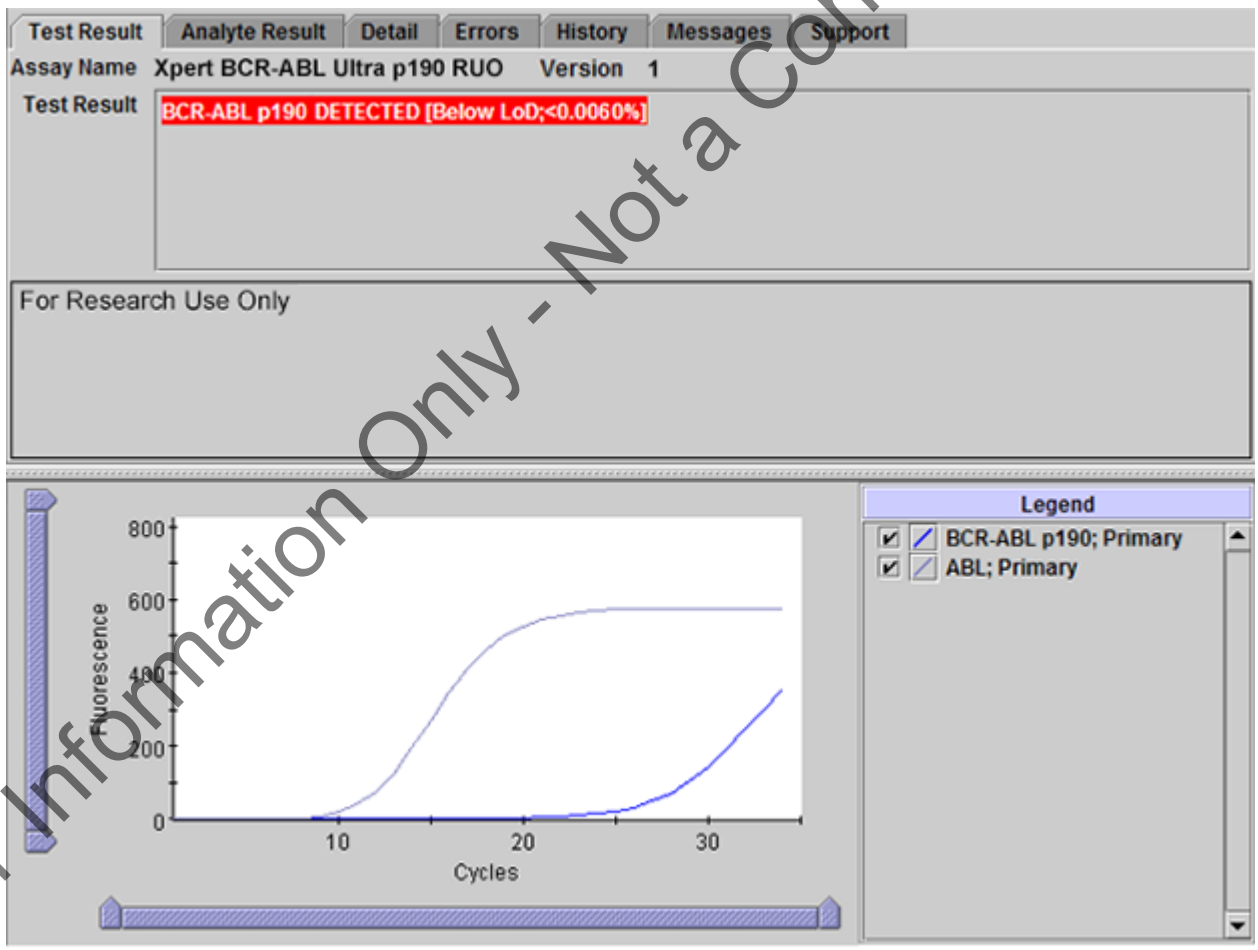

Figure 4. GeneXpert Dx View Results Window: BCR-ABL p190 DETECTED [Below LoD; <0.0060%]

15.4 BCR-ABL p190 NOT DETECTED [Sufficient ABL transcript]

BCR-ABL p190 was not detected with BCR-ABL p190 Ct equal to “0” or greater than the cut-off of “32” and ABL Ct greater than “8” and less than or equal to “18”.

When BCR-ABL p190 is undetectable with BCR-ABL p190 Ct equal to “0” or greater than the cut-off of “32”, the GeneXpert software first looks for the ABL Ct to confirm if the ABL Ct is greater than or equal to “8” and less than or equal to “18” to ensure having “Sufficient ABL transcript”. See Table 2.

Example: Assay’s BCR-ABL p190 Ct = 0; ABL Ct = 11.6 is less than “18”.

Result: **BCR-ABL p190 NOT DETECTED [Sufficient ABL transcript]**. See Figure 5.

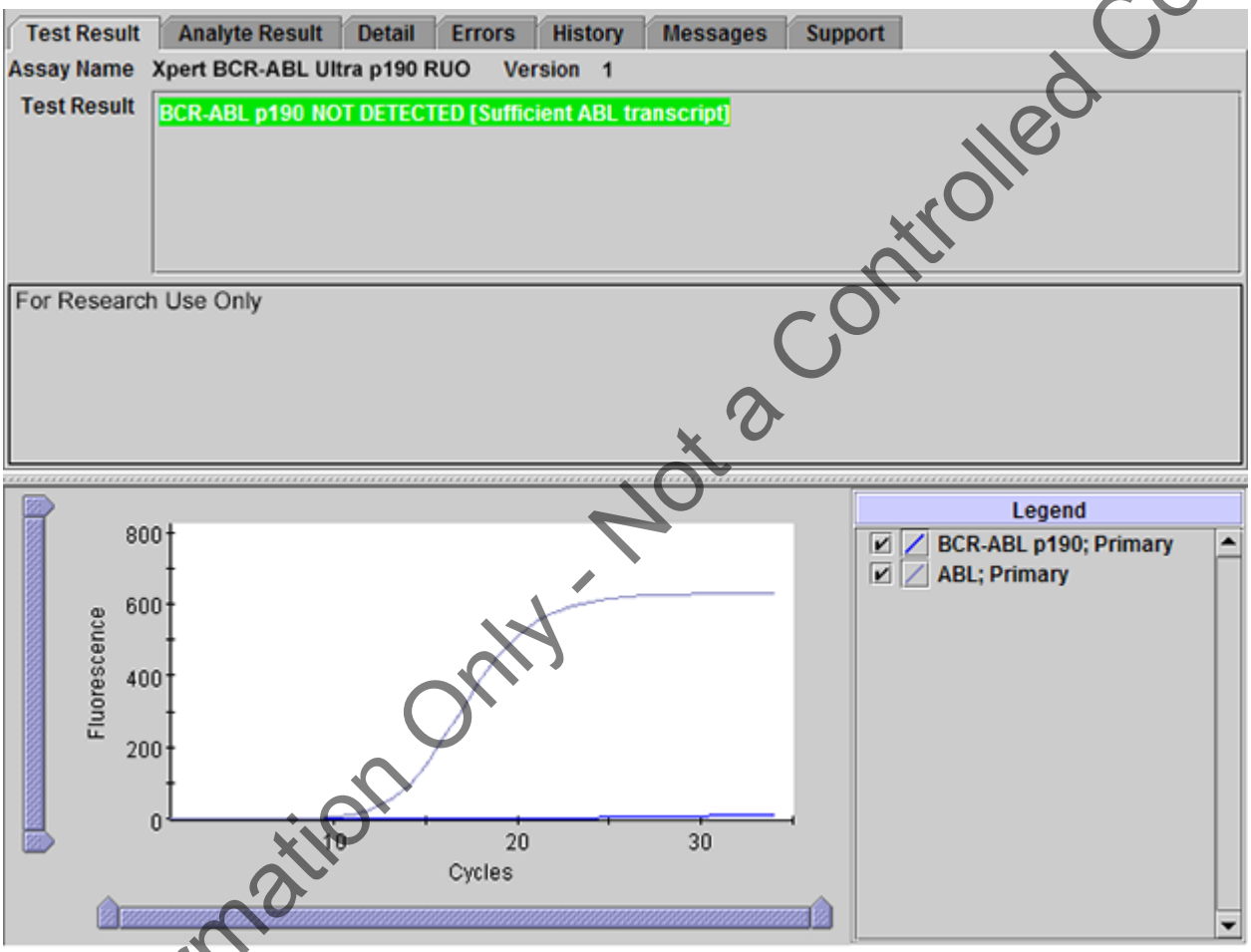

Figure 5. GeneXpert Dx View Results Window: BCR-ABL p190 NOT DETECTED [Sufficient ABL transcript]

15.5 INVALID [No ABL transcript]

BCR-ABL p190 was not detected with ABL Ct equal to "0".

When BCR-ABL p190 is either detected or not detected, the GeneXpert software first looks for the ABL Ct to confirm if the ABL Ct is less than or equal to "18" to ensure having "Sufficient ABL transcript". Refer to Section 17, Troubleshooting Guide.

Example: Assay's BCR-ABL p190 Ct = 0; ABL Ct = 0.

Result: **INVALID [No ABL transcript]**. See Figure 6.

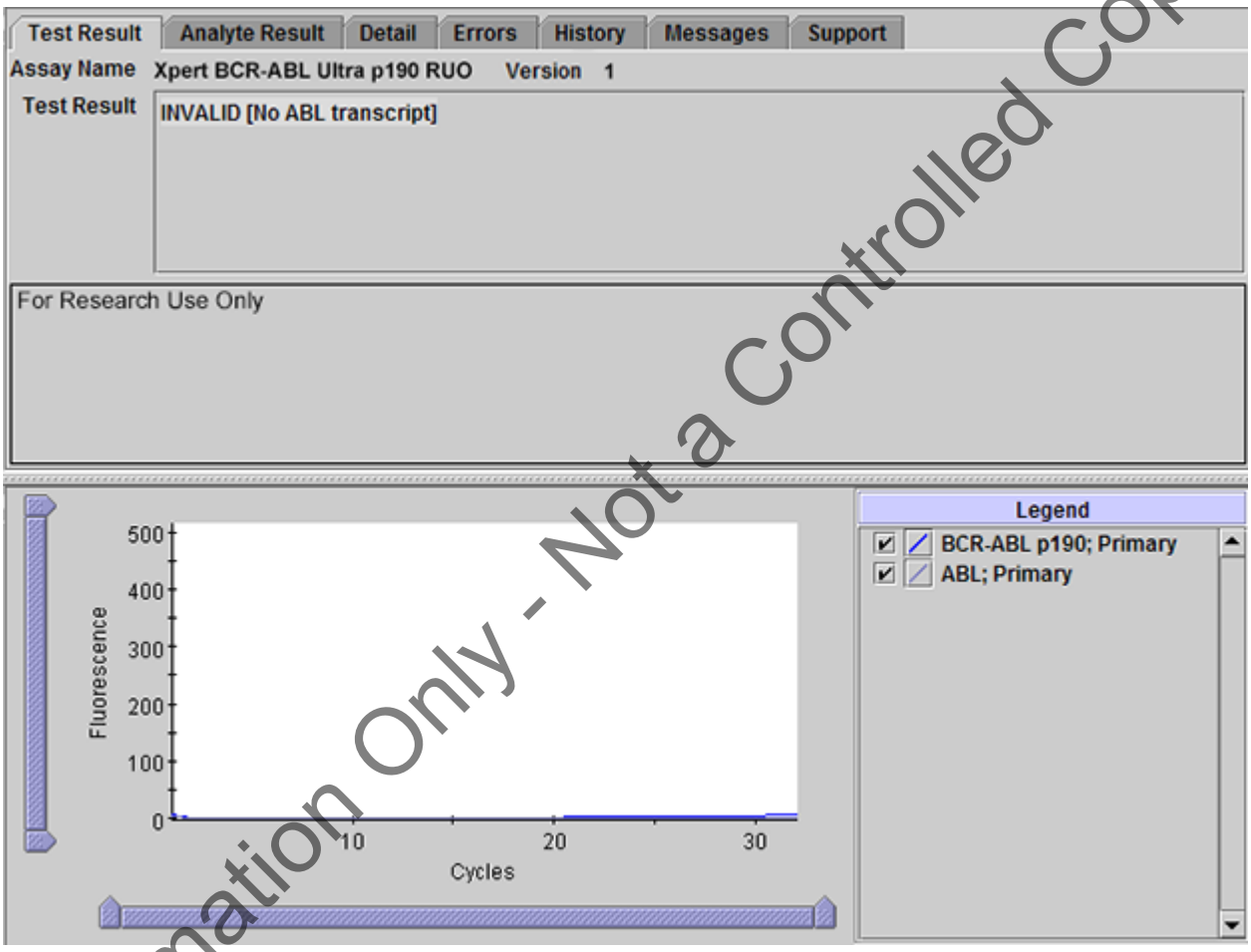

Figure 6. GeneXpert Dx View Results Window: INVALID [No ABL transcript]

15.6 INVALID [Insufficient ABL transcript]

BCR-ABL p190 was not detected with ABL Ct greater than "18".

When BCR-ABL p190 is either detected or not detected, the GeneXpert software first looks for the ABL Ct to confirm if the ABL Ct is less than or equal to “18” to ensure having “Sufficient ABL transcript”. Refer to Section 17, Troubleshooting Guide.

Example: Assay’s BCR-ABL p190 Ct = 31.2; ABL Ct = 28 is greater than "18".

Result: **INVALID [Insufficient ABL transcript]**. See Figure 7.

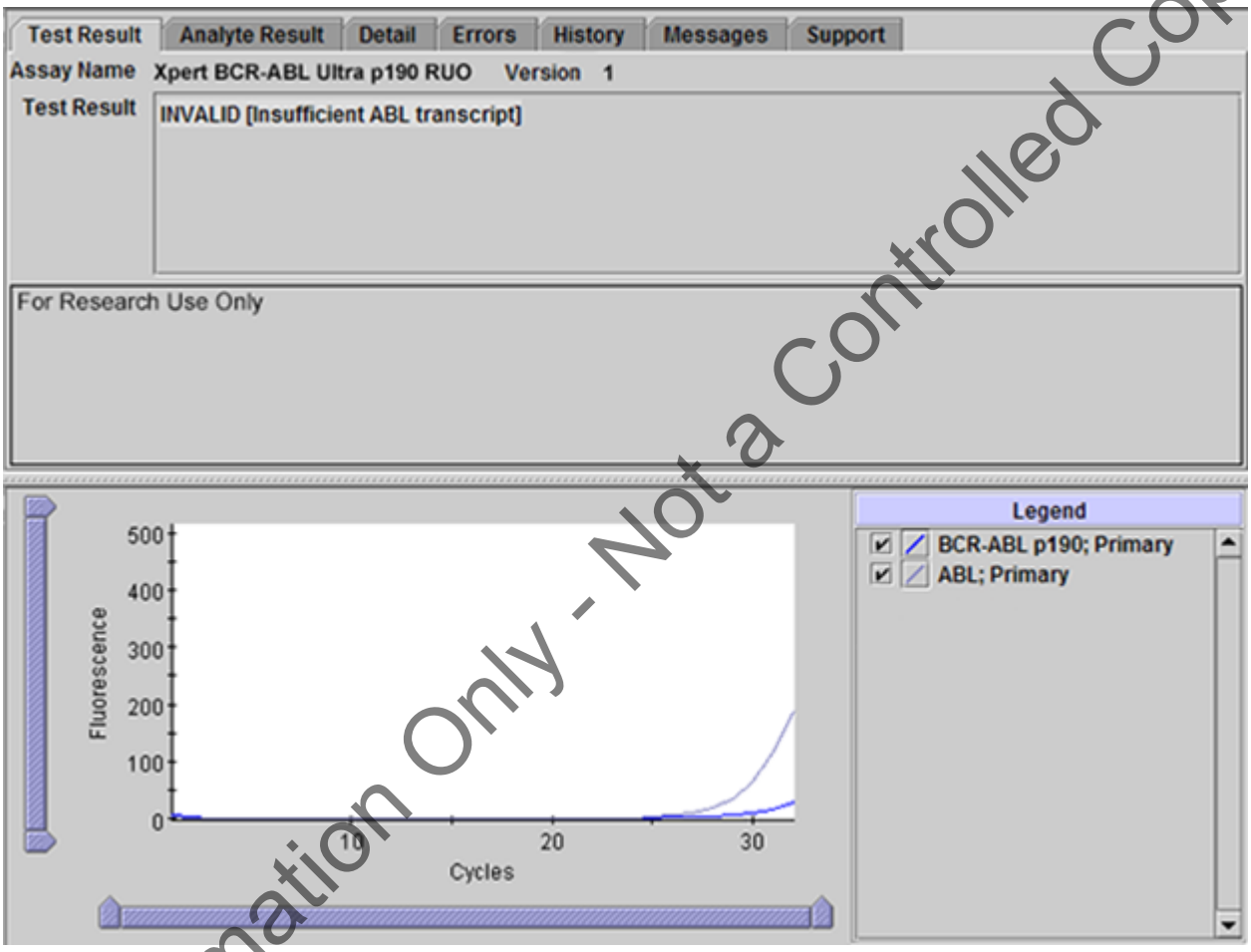

Figure 7. GeneXpert Dx View Results Window: INVALID [Insufficient ABL transcript]

15.7 INVALID [Too high BCR-ABL p190 and ABL transcripts]

BCR-ABL p190 was detected with both BCR-ABL p190 and ABL Cts less than "8".

When BCR-ABL p190 is either detected or not detected, the GeneXpert software first looks for the ABL Ct to confirm if the ABL Ct is less than or equal to "18" to ensure having "Sufficient ABL transcript". Refer to Section 17, Troubleshooting Guide.

Example: Assay's BCR-ABL p190 Ct = 7.9; ABL Ct = 7.6 is less than "8".

Result: **INVALID [Too high BCR-ABL p190 and ABL transcripts]**. See Figure 8.

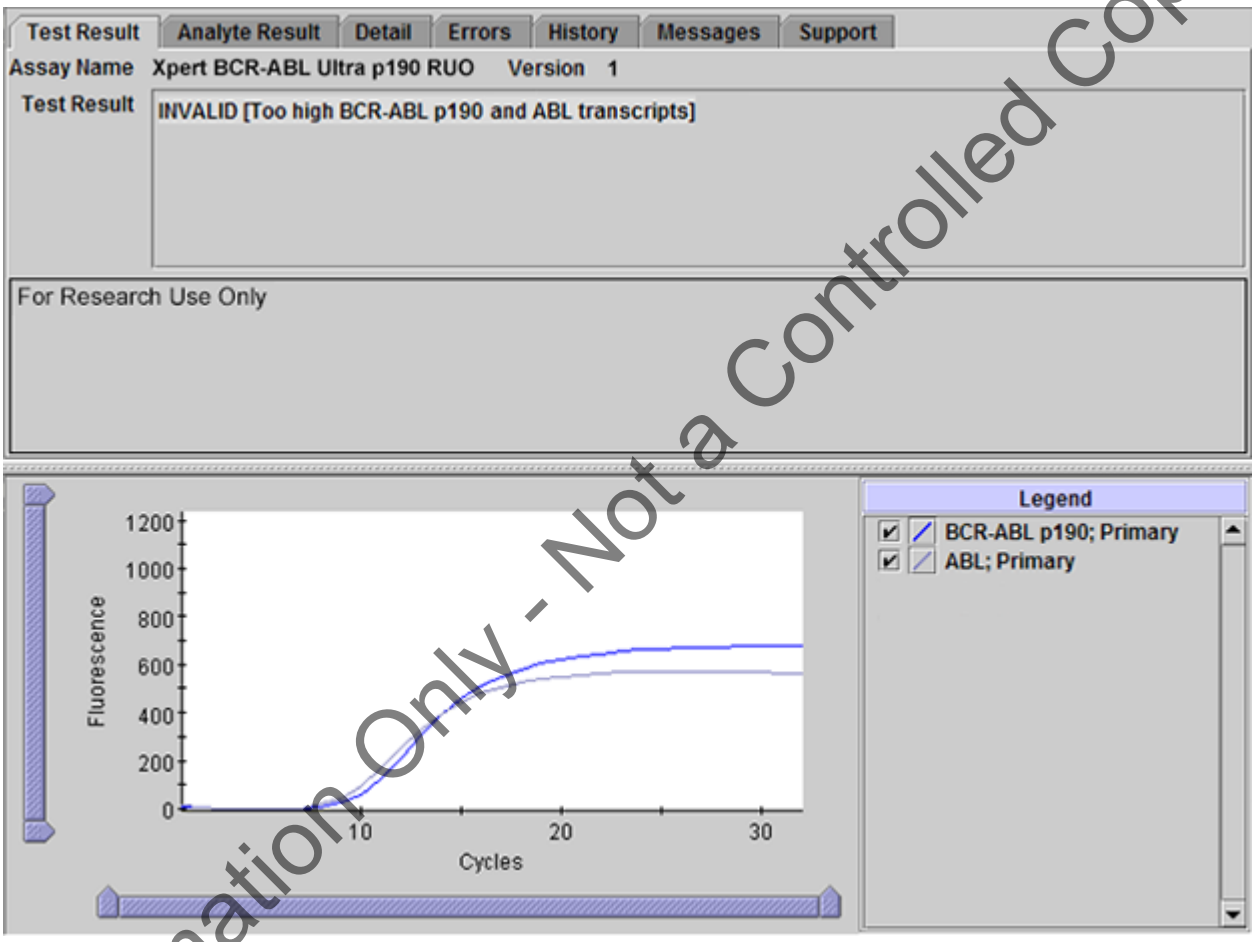

Figure 8. GeneXpert Dx View Results Window: INVALID [Too high BCR-ABL p190 and ABL transcripts]

15.8 ERROR

Figure 9. GeneXpert Dx View Results Window: ERROR

16 Limitations of the Assay

- For Research Use Only. Not for Use in Diagnostic Procedures.
- The assay is not intended to be used with external calibrators.
- Modifications to these procedures may alter the function of the assay.
- This product was designed for use with blood collected in EDTA tubes only.
- Do not use heparin as the anticoagulant because it can inhibit the PCR reaction.
- Sodium citrate (NaCitrate), buffy-coat and bone marrow sample types have not been validated.
- Erroneous assay results might occur from improper sample collection, handling or storage or sample mix-up. Careful compliance to the instructions in this package insert is necessary to avoid erroneous results.
- Samples with high white blood cell counts (greater than 30 million cells/mL) tested per Section 11.2.1 may yield inaccurate results, **INVALID** results, or aborted runs due to pressure build-up within the cartridge. See Table 3 for additional information.
- Samples with very low levels of ABL transcript or with white blood cells lower than 150,000 cells/mL may be reported as **INVALID** (Type 1). A non-determinate result does not preclude the presence of very low levels of leukemic cells in the sample.
- Mutations or polymorphisms in primer or probe binding regions may affect detection of new or unknown variants and may result in a false negative result.

17 Troubleshooting Guide

Table 3. Troubleshooting Guide

Assay Result	Possible Causes	Suggestions
INVALID	Type 1: Endogenous control ABL failure: ● Poor sample quality ● RT-PCR inhibition ● If ABL Ct > 18, and/or endpoint <200	● Check the sample quality (e.g., exceeded sample storage requirement including time and temperature). ● Repeat the assay with original sample (if available) or from retained lysate and a new cartridge following the procedure as described in Section 18.1, Retest Procedure for ERROR or INVALID (Type 1).
	Type 2: BCR-ABL p190 transcript level cannot be determined due to sample containing excess BCR-ABL p190 and/or ABL transcripts (Ct < 8)	Repeat the assay with original sample (if available) or from retained lysate and a new cartridge following the procedure as described in Section 18.2, Retest Procedure for ERROR (Code 2008) or INVALID (Type 2).
ERROR (Code 2008)	Pressure exceeding limit (error message 2008)	● Check the sample quality ● Check for grossly elevated WBC count ● Repeat the assay with original sample (if available) or from retained lysate and a new cartridge following the procedure as described in Section 18.2, Retest Procedure for ERROR (Code 2008) or INVALID (Type 2).
ERROR (Code 5006, 5007, 5008, 5009*) *This is not an exhaustive list of ERROR codes.	Probe check failure	Repeat the assay with original sample (if available) or from retained lysate and with a new cartridge following the procedure as described in Section 18.1, Retest Procedure for ERROR or INVALID (Type 1).
NO RESULT	Data collection failure. For example, the operator stopped an assay that was in progress or a power failure occurred.	Repeat the assay with original sample (if available) or from retained lysate and with a new cartridge following the procedure as described in Section 18.1, Retest Procedure for ERROR or INVALID (Type 1).

18 Retests

18.1 Retest Procedure for ERROR or INVALID (Type 1)

Retest samples with **ERROR** or **INVALID** results due to the ABL cycle threshold (Ct) exceeding the maximum valid Ct cut-off (Ct >18) or the endpoint is below the threshold setting (<200). Also refer to Section 17, Troubleshooting Guide.

1. If sufficient blood sample volume is available, re-test from original blood sample collection tube following the procedure in Section 11.2, Preparing the Sample.

   -OR-

   If blood sample volume is insufficient, re-test can be performed with the retained lysate from Section 11.2, Preparing the Sample, Step 12.

   a. If retained lysate from Section 11.2, Preparing the Sample, Step 12 is stored frozen, thaw to room temperature before use.
   b. Ensure lysate is well-mixed by mixing the sample with a vortex mixer at maximum setting continuously for 10 seconds and set it aside for 3 minutes for bubbles to settle. Go to Section 11.2, Preparing the Sample, Step 13.
2. Transfer 1 mL of the prepared lysate into a new 50 mL conical tube.
3. To the new conical tube containing lysate, add 1.5 mL of Lysis Reagent (LY).
4. Follow Steps 3-13 in Section 11.2.2 to make the final lysate.
5. Open the cartridge by lifting the cartridge lid and transfer the entire contents of the Wash Reagent (1) ampoule to the Wash Reagent chamber (with small opening). See Figure 1.
6. Pipette the entire contents of the prepared sample into the Sample Chamber (large opening). See Figure 1.
7. Close cartridge lid. Initiate assay (see Section 11.4, Starting the Assay).

18.2 Retest Procedure for ERROR (Code 2008) or INVALID (Type 2)

Retest samples with BCR-ABL p190 and/or ABL transcript levels below the valid minimum Ct cut-off (Ct<8) and/or when pressure limit is exceeded. Also refer to Section 17, Troubleshooting Guide.

1. To the bottom of a new 50 mL conical tube, add 100µL of PK (Proteinase K).
2. Ensure blood sample or left-over lysate from Section 11.2, Preparing the Sample, Step 12 is well-mixed by inverting the tube 8 times immediately before pipetting.
3. To the tube already containing Proteinase K, add 50 µL of blood sample, if available, or 80 µL of left-over lysate from Section 11.2, Preparing the Sample, Step 12.

   a. Mix the sample with a vortex mixer at maximum setting continuously for 3 seconds.
   b. Incubate at room temperature for 1 minute.
4. To the new conical tube containing lysate, add 2.5 mL of Lysis Reagent (LY).
5. Mix the sample with a vortex mixer at maximum setting continuously for 10 seconds.
6. Incubate at room temperature for 5 minutes.
7. Mix the sample with a vortex mixer at maximum setting continuously for 10 seconds.
8. Incubate at room temperature for 5 minutes.
9. To the same conical tube, add 2 mL of reagent grade absolute ethanol (not provided).
10. Mix the sample with a vortex mixer at maximum setting continuously for 10 seconds.
11. Open the cartridge by lifting the cartridge lid and transfer the entire contents of the Wash Reagent (1) ampoule to the Wash Reagent chamber (with small opening). See Figure 1.
12. Pipette the entire contents of the prepared sample into the Sample Chamber (large opening). See Figure 1.
13. Close cartridge lid. Initiate assay (see Section 11.4, Starting the Assay).

19 Analytical Data

The data was collected from internal studies only.

19.1 Assay Linearity/Dynamic Range

The linearity of the assay was evaluated by serially diluting total RNA isolated from SUP-B15 (e1a2) cell line in a background lysate prepared from normal EDTA peripheral blood samples to target 9 different levels ranging between 25% to 0.001% BCR-ABL p190/ABL and testing each level, including the negative, on two assay kit lots for a total of 80 replicates.

Linear regression analyses were performed for first, second and third order polynomials. The results were considered linear if the polynomial regression coefficients were insignificant (*p*-values > 0.05). The combined linear regression curve for e1a2 transcript from two kit lots is shown in Figure 10.

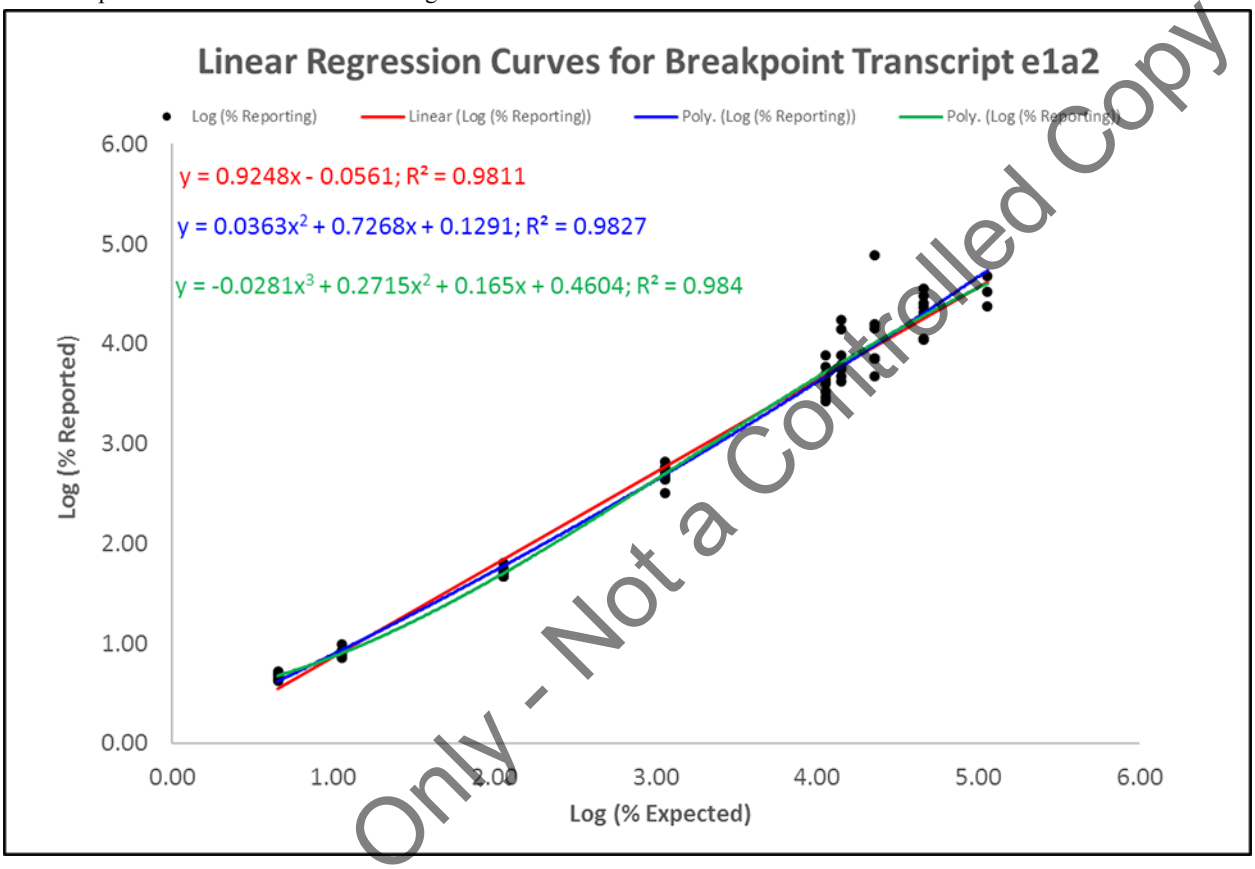

Figure 10. Linear Regression Curves for p190 Transcript (e1a2)

The estimated regression intercept, slope, and R^2 value from the linear model are shown in Table 4.

Table 4. Regression Coefficients from Linear Model

Intercept	Slope	R^2
-0.0561	0.9248	0.9811

19.2 Assay Sensitivity

The analytical limit of blank (LoB) was established using 40 normal EDTA peripheral blood samples. Two out of 130 total replicates tested reported as BCR-ABL p190 **DETECTED**. The overall LoB was determined to be 0.00031%.

The analytical limit of detection (LoD) was estimated for the p190 transcript using dilutions of SUP-B15 cell line in negative EDTA blood background. The estimated analytical LoD is the lowest analyte concentration (reported as % BCR-ABL p190/ ABL transcript) that can be reliably distinguished from the LoB > 95% of the time. The estimated analytical LoD of the assay is 0.0060% BCR-ABL p190/ABL.

The limit of quantitation (LoQ) was estimated with the data obtained from the LoD study. The mean for the % values was calculated for replicates at levels equal to the LoD, 0.0060%, or greater with positivity greater or equal to 95%. The LoQ of the assay is constrained by the LoD of the assay; therefore, the LoQ was determined to be equal to the LoD, 0.0060%.

20 References

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6. Centers for Disease Control and Prevention. Biosafety in Microbiological and Biomedical laboratories (refer to latest edition). http://www.cdc.gov/biosafety/publications/
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8. https://www.who.int/news-room/fact-sheets/detail/health-care-waste

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22 Technical Assistance

Before contacting Cepheid Technical Support, collect the following information:

- Product name
- Lot number
- Serial number of the instrument
- Error messages (if any)
- Software version and, if applicable, Computer Service Tag number

United States Technical Support

Telephone: + 1 888 838 3222
Email: techsupport@cepheid.com

Contact information for all Cepheid Technical Support offices is available on our website: www.cepheid.com/en/support/contact-us.

23 Table of Symbols

Symbol	Meaning
REF	Catalog number
RUO	Research Use Only
LOT	Batch code
2	Do not reuse
	Consult instructions for use
	Manufacturer
CC	Country of manufacture
Σ	Contains sufficient for *n* tests
CONTROL	Control
	Expiration date
°C	Temperature limitation
	Biological risks
	Caution
	Warning

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24 Revision History

Description of Changes: 302-3770 Rev. A to Rev. B

Purpose: Update per core team revisions

Section	Description of Change
All	New standard formatting updates and minor adjustments throughout package insert
Trademark page	Updates to copyright claim date format and statements per legal requirements
Section 4	Replaced "patients" with "individuals" in multiple locations
Section 11.2.1	New heading
Section 11.2.2	New section
Section 16	Inserted additional limitations statements
Section 18.1	Removed steps and added a step that directs users to new section (Section 11.2.2) for procedure
Section 24	Insert revision history table