

# Instructions for Use:

## Bloodhound™ BCR::ABL1 Quantitative Reagents

For Quantification of BCR::ABL1 p210, p190, p230, and p203 transcripts.

**RUO** For research use only. Not for use in diagnostic procedures.

**Please read the Instructions for Use thoroughly before you use this product and keep it for future reference.**

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**BH-20R-BCR, BH-4P-BCR, BH-8P-BCR, BH-12P-BCR**



**Precipio, Inc. 4 Science Park, New Haven, CT 06511**

**NOTE: Internal optimization recommended**

## Intended Use

For research use only (RUO). Not for use in diagnostic procedures. This reagent set is specifically designed to be used by suitably trained personnel following internal optimization of methods and analysis procedures. The use of this product is not intended to provide information for the diagnosis, prevention, or treatment of a disease.

## Indications for Use

Bloodhound™ BCR::ABL1 Reagents detect three major BCR::ABL1 isoforms (p190, p210, and p230) and one minor isoform (p203) associated with BCR-ABL1-induced leukemogenesis, including chronic myeloid leukemia (CML) and acute lymphocytic leukemia (ALL). The presence of fusion isoforms can be associated with diagnosis, therapeutic monitoring, detection of minimal residual disease (MRD), therapeutic selection for response to TKIs, and/or stem cell transplantation. The results from Bloodhound BCR::ABL1 are quantitative.

## Biological Reference

BCR::ABL1 is a tumor marker for Philadelphia (Ph) chromosome positive leukemia. The status of the BCR::ABL1 fusion leads to informed prognosis and clinical management decisions and serves as a paradigm for the successful application of molecularly targeted cancer therapy. <sup>(1-3)</sup>

The BCR::ABL1 fusion gene results from the usually reciprocal chromosomal translocation t(9;22)(q34;q11) (Philadelphia chromosome) of two genes, ABL on chromosome 9 (a cellular proto-oncogene coding for a tyrosine kinase), and BCR on chromosome 22 (which codes for a serine kinase). In leukemia cells, the Ph chromosome impairs the survival and proliferation signaling pathways and disrupts genomic stability. The Ph chromosome is a hallmark of CML and is associated with 95% of CML cases and approximately 25% of adult ALL cases and less frequently in other hematological diseases. <sup>(1)</sup>

## Fusion Isoforms: p210, p190, p230, and p203

Bloodhound™ BCR::ABL1 Reagents detect multiple variations of four common BCR::ABL1 isoforms p210, p190, p230, and p203. Each isoform is produced by a fusion of the BCR and ABL genes but differs in the location of the breakpoint, which determines which BCR::ABL1 protein isoforms are expressed, and are linked to different disease phenotypes. <sup>(4-6)</sup>

Bloodhound BCR::ABL1 covers four variants of isoforms p210 and p203 between BCR exons 13 and 14 (b2 and b3) and ABL exons 2 and 3(a2 and a3), two variants of isoform p190 between BCR exons 1 and ABL exons 1 and 2(e1 and a2) and exon 3(a3), and two variants of isoform p230 between BCR exon 19 (e19) and ABL exons 2 and 3(a2 and a3) (**Figure 1**).

The p210 (b2a2 or b3a2) isoform (M-BCR), classified as the major breakpoint transcript, is located between exons 13 and 14 in BCR. The presence of the p210 fusion is a molecular marker of chronic myelogenous leukemia (CML) in the stable phase and is associated with durable remission when treated with TKIs. The p210 isoform is found in 25% of B-ALL patients and 3% of AML patients. <sup>(7 & 8)</sup>

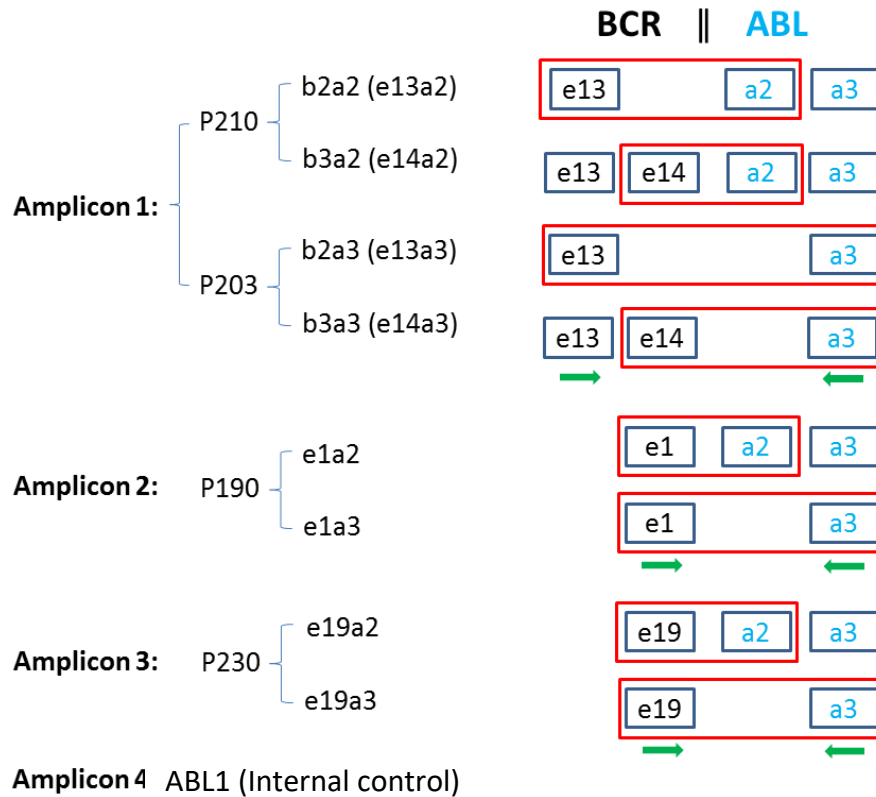
The p190 isoform (m-BCR), classified as the minor breakpoint transcript, occurs within BCR exon 1 and is associated with increased monocytosis and absence of basophilia. Isoform p190 exhibits stronger kinase activity compared to isoform p210 and has a tendency to progress to the lymphoid blast phase. The fusion isoform is present in approximately 75% of adult B-ALL, where relapse and TKI resistance is frequent, and overall survival is low. <sup>(7 & 8)</sup>

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Isoform p230 is located between BCR exons 19 and 20 and produces a fusion protein,  $\mu$ -BCR, which contains additional BCR coding sequences that are not found in the p190 or p210 variants. Isoform p230 is associated with increased neutrophilic maturation or thrombocytosis and a milder course of CML.<sup>(9)</sup>

Isoform p203 (b3a3/e14a3 and b2a3/e13a3) is formed with the fusion of BCR breakpoint within exons 13 or 14 with ABL exon 3 instead of the more common exon 2.<sup>(5 & 6)</sup> Isoform p203 is rarely seen in CML.<sup>(10 & 11)</sup> Rare BCR::ABL1 fusion transcripts may be underreported due to lack of available testing or detection of typical fusion transcripts.

**Figure 1: Schematic diagram of each Bloodhound BCR::ABL1 assay coverage. Each assay covers a set of the BCR::ABL1 fusion genes, and the approximate locations of the BCR and ABL1 breakpoints are provided.**



## Principles of Procedure

The presence of a BCR::ABL1 fusion variant is indicated through amplification of target sequences in the presence of high fluorescence dsDNA-binding dye and melt curve analysis. Forward and Reverse primers hybridize to specific sequences and only amplify in the presence of a fusion product. If no fusion product exists, no production of a large fusion product will occur. Immediately after amplification, melt curve analysis measures the fluorescence of each product in the reaction as the temperature increases from 65 °C to 95 °C and will indicate if a product was amplified and identify the melting temperature ( $T_m$ ) of each product. Each assay contains an amplification control to confirm that each reaction occurred as expected and interpretation of the fluorescent signal will indicate the presence or absence of a fusion.

In addition, the Ct values observed can provide quantitative information on the amount of the translocation present within the original sample as compared to an internal ABL1 control.

## Instructions for Use

### Traceability of Bloodhound™ BCR::ABL1 Reagents Controls

The “Wild-Type Control” and “Mutant Control” sequences were constructed by synthesis and cloning using the NCBI Reference Sequences:

NM\_005157.6 *ABL1*  
NM\_004327.4 *BCR*

### Materials Required but Not Provided

#### Reagents

- RNA Isolation Kit
- Reagents for reverse transcription
- MeltDoctor™ Calibration Plate

#### Consumables

- Nuclease-free, filtered, sterile PCR pipette tips
- 1.5 mL capped nuclease-free PCR tubes
- 0.1 mL Fast PCR plates
- Optical plate seals

#### Equipment Needed

- Mechanical Pipettes 1-200 µL dedicated for PCR
- Mini Centrifuge with rotor for 1.5-2.0 mL tubes
- Microfuge capable of holding respective PCR plate (96-well or 384-well)
- A high caliber qPCR instrument such as the Applied Biosystems QuantStudio™ 3, 5, 6, 7 or 7500 Real-time PCR systems with a 0.1 mL Fast Heat Block and the associated instrument specific materials.

### Materials Provided

The Bloodhound™ BCR::ABL1 Reagents are supplied as ready to use. Bloodhound™ BCR::ABL1 Reagents can be purchased as free-flow or pre-plated. Pre-plated reagents come in the following layouts: 4 samples in duplicate, 8 samples in duplicate, and 12 samples in duplicate. The free-flow reagents come as 20-samples in duplicate, see table 1 for a detailed list of set contents.

The Bloodhound™ BCR::ABL1 Reagents require the user to run a calibration plate prior to use of the assay. The calibration plate is a one-time use plate, that is shipped ready for use. It is recommended the calibration plate be run every 6 months.

**NOTE:** Any major machine maintenance including, but not limited to, replacement of the bulb, replacement of filters, and replacement of the heat block requires the instrument to be re-calibrated.

## Instructions for Use

**Table 1: Reagent Set Contents**

Vial	Volume	Coverage
p210/p203 Assay*	1020 µL	p210 b2a2 (e13a2), p210 b3a2 (e14a2), p203 b2a3 (e13a3), p203 b2a3 (e14a3)
p190 Assay*	1020 µL	p190 (e1a2) p190 (e1a3)
p230 Assay *	1020 µL	p230 (e19a2) p230 (e19a3)
ABL1 Assay	1020 µL	Copy Number Detection
ABL1 Control	20 µL	-
p210 Positive Control	20 µL	-
p203 Positive Control	20 µL	-
p190 Positive Control	20 µL	-
p230 Positive Control	20 µL	-

\*All assay mixes contain an additional internal control amplicon

### Pre-plated layouts:

4-samples in duplicate:

1	2	3	4	5	6	7	8	9	10	11	12
p210_p203, R1, S1	p210_p203, R2, S1	p190, R1, S1	p190, R2, S1	p230, R1, S1	p230, R2, S1	IC CTRL, S1			p210 CTRL		p210_p203 NTC
p210_p203, R1, S2	p210_p203, R2, S2	p190, R1, S2	p190, R2, S2	p230, R1, S2	p230, R2, S2	IC CTRL, S2			p203 CTRL		p190 NTC
p210_p203, R1, S3	p210_p203, R2, S3	p190, R1, S3	p190, R2, S3	p230, R1, S3	p230, R2, S3	IC CTRL, S3			p190 CTRL		p230 NTC
p210_p203, R1, S4	p210_p203, R2, S4	p190, R1, S4	p190, R2, S4	p230, R1, S4	p230, R2, S4	IC CTRL, S4			p230 CTRL		IC NTC
									IC CTRL		RT IC NTC (Optional)

8-samples in duplicate:

	1	2	3	4	5	6	7	8	9	10	11	12
A	p210_p203, R1, S1	p210_p203, R2, S1	p190, R1, S1	p190, R2, S1	p230, R1, S1	p230, R2, S1	IC CTRL, S1			p210 CTRL		p210_p203 NTC
B	p210_p203, R1, S2	p210_p203, R2, S2	p190, R1, S2	p190, R2, S2	p230, R1, S2	p230, R2, S2	IC CTRL, S2			p203 CTRL		p190 NTC
C	p210_p203, R1, S3	p210_p203, R2, S3	p190, R1, S3	p190, R2, S3	p230, R1, S3	p230, R2, S3	IC CTRL, S3			p190 CTRL		p230 NTC
D	p210_p203, R1, S4	p210_p203, R2, S4	p190, R1, S4	p190, R2, S4	p230, R1, S4	p230, R2, S4	IC CTRL, S4			p230 CTRL		IC NTC
E	p210_p203, R1, S5	p210_p203, R2, S5	p190, R1, S5	p190, R2, S5	p230, R1, S5	p230, R2, S5	IC CTRL, S5			IC CTRL		RT IC NTC (Optional)
F	p210_p203, R1, S6	p210_p203, R2, S6	p190, R1, S6	p190, R2, S6	p230, R1, S6	p230, R2, S6	IC CTRL, S6					
G	p210_p203, R1, S7	p210_p203, R2, S7	p190, R1, S7	p190, R2, S7	p230, R1, S7	p230, R2, S7	IC CTRL, S7					
H	p210_p203, R1, S8	p210_p203, R2, S8	p190, R1, S8	p190, R2, S8	p230, R1, S8	p230, R2, S8	IC CTRL, S8					

## Instructions for Use

12-samples in duplicate:

	1	2	3	4	5	6	7	8	9	10	11	12
A	p210_p203, R1, S1	p210_p203, R1, S2	p210_p203, R1, S3	p210_p203, R1, S4	p210_p203, R1, S5	p210_p203, R1, S6	p210_p203, R1, S7	p210_p203, R1, S8	p210_p203, R1, S9	p210_p203, R1, S10	p210_p203, R1, S11	p210_p203, R1, S12
B	p210_p203, R2, S1	p210_p203, R2, S2	p210_p203, R2, S3	p210_p203, R2, S4	p210_p203, R2, S5	p210_p203, R2, S6	p210_p203, R2, S7	p210_p203, R2, S8	p210_p203, R2, S9	p210_p203, R2, S10	p210_p203, R2, S11	p210_p203, R2, S12
C	p190, R1, S1	p190, R1, S2	p190, R1, S3	p190, R1, S4	p190, R1, S5	p190, R1, S6	p190, R1, S7	p190, R1, S8	p190, R1, S9	p190, R1, S10	p190, R1, S11	p190, R1, S12
D	p190, R2, S1	p190, R2, S2	p190, R2, S3	p190, R2, S4	p190, R2, S5	p190, R2, S6	p190, R2, S7	p190, R2, S8	p190, R2, S9	p190, R2, S10	p190, R2, S11	p190, R2, S12
E	p230, R1, S1	p230, R1, S2	p230, R1, S3	p230, R1, S4	p230, R1, S5	p230, R1, S6	p230, R1, S7	p230, R1, S8	p230, R1, S9	p230, R1, S10	p230, R1, S11	p230, R1, S12
F	p230, R2, S1	p230, R2, S2	p230, R2, S3	p230, R2, S4	p230, R2, S5	p230, R2, S6	p230, R2, S7	p230, R2, S8	p230, R2, S9	p230, R2, S10	p230, R2, S11	p230, R2, S12
G	IC CTRL, S1	IC CTRL, S2	IC CTRL, S3	IC CTRL, S4	IC CTRL, S5	IC CTRL, S6	IC CTRL, S7	IC CTRL, S8	IC CTRL, S9	IC CTRL, S10	IC CTRL, S11	IC CTRL, S12
H	p210 CTRL	p203 CTRL	p190 CTRL	230 CTRL	IC CTRL			p210_p203 NTC	p190 NTC	p230 NTC	IC NTC	RT IC NTC (Optional)

## Storage and Handling

- Reagent sets are shipped on dry ice and must be stored upon receipt in the dark between  $-20\text{ }^{\circ}\text{C} \pm 2^{\circ}\text{C}$  in a constant temperature freezer until use.
- Store all free-flow set components in the original containers. Do not aliquot.
- Minimize exposure of the pre-plated and free-flow reagents to light.
- If using free-flow set, gently mix and centrifuge the tubes before opening.
- Ensure all reagents (free-flow and pre-plated) are stored properly sealed, in the correct up-right position. Pre-plated reagent packaging indicates the correct orientation of the plates.
- Note the expiration date of each reagent set received. Do not use it after the expiration date.

## Sample Preparation

The quality of the assay is highly dependent on the quality of input RNA. Sample processing should be performed using a standardized protocol and the quality of the RNA should be verified before use.

1. Initial RNA used should be between 0.5 and 1  $\mu\text{g}$ . No less than 20  $\text{ng}/\mu\text{L}$  of initial starting RNA. If yield is less than 20  $\text{ng}/\mu\text{L}$ , re-extraction is necessary. Internal optimization required.
2. It is recommended that all samples be extracted with the same protocol to avoid any variations due to reagents/buffers. If utilizing free-flow reagents, it is recommended an additional NTC goes through the cDNA process as well, to be plated with the extracted specimens.
3. Ensure sample-to-sample uniformity.

## Procedure

### Recommendations before you start

The following procedures are optional but highly recommended prior to PCR setup:

1. Turn on UV light inside the hood (15 min).
2. UV crosslink empty tubes.
3. Prepare workspaces for analysis. Wipe all dedicated pipettes, tips, and equipment with decontaminating solutions such as 70% ethanol, RNase AWAY® or RNaseZap™ solution.
4. BCR::ABL1 calibration plate must be run prior to validation. The BCR::ABL1 calibration plate is provided by Precipio and will be essential for analysis.

## Instructions for Use

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### Procedure for Running the Calibration Plate

The calibration plate is used to determine instrument specific fluorescence values, based on known digitally verified controls. The calibration plate accounts for instrument variables such as light bulb brightness, instrument age, and heat block distribution. The plate is ready to use, and is a one-time use plate.

**NOTE:** Any major machine maintenance including, but not limited to, replacement of the bulb, replacement of filters, and replacement of the heat block requires the instrument to be re-calibrated.

1. Thaw the calibration plate.
2. Thaw the plate for a minimum of 20 minutes. Once thaw centrifuge for approximately 1-2 mins (5,000-10,000 rpm) to collect the liquid in the bottom of the plate.
3. Ensure the seal is properly secured on the plate.
4. Place the plate in the qPCR instrument, ensuring the plate is properly orientated.
5. Perform the run method on the qPCR instrument according to **Table 2**.

### Procedure for Free-Flow Reagents

1. Thaw all necessary components.
2. Briefly vortex the assay mix and centrifuge for approximately 3-10 sec (5,000-10,000 rpm) to collect the liquid in the bottom of the tube.
3. Plate 20  $\mu$ L of each premixed primer into each reaction well in a 96-well PCR reaction plate.
4. Add 3  $\mu$ L of the cDNA product to each reaction. Mix gently by pipetting up and down.
  - a. The same sample should be added to two separate wells for each assay for replicate analysis.
5. Add 3  $\mu$ L of control into the designated reaction wells.
6. Add 3  $\mu$ L of NTC into the designated reaction wells.

**OPTIONAL:** Add 3  $\mu$ L of the cDNA negative control to the appropriate well (RT IC NTC). This is the same NTC that has gone through the cDNA process with the specimens. Omit if not required.

7. Place an optical seal over the plate and centrifuge (5,000-10,000 rpm, approximately 30 sec). Inspect the plate for any air bubbles and well uniformity prior to placing the plate in the qPCR instrument.
8. Perform the run method on the qPCR instrument according to **Table 2**.



## Instructions for Use


### Procedure for Pre-plated Reagents:

1. Thaw all necessary components.
  - i Remove the packaging from the freezer and store in a dark space for a minimum of 40 minutes.
  - ii Once the 40 minutes have passed, open the external packaging, remove the plate and centrifuge the plate for 2 minutes at 5,000-10,000 rpm.
2. Add 3  $\mu$ L of the cDNA product to each reaction. Mix gently by pipetting up and down.
3. The same sample should be added to two separate wells for each assay for replicate analysis.

**OPTIONAL:** Add 3  $\mu$ L of the cDNA negative control to the appropriate well (RT IC NTC). This is the same NTC that has gone through the cDNA process with the specimens. Omit if not required.

4. Place an optical seal over the plate and centrifuge (>300 x g, approximately 30 sec). Inspect the plate for any air bubbles and well uniformity prior to placing the plate in the qPCR instrument.
5. Perform the run method on the qPCR instrument according to **Table 2**.

### Amplification and Melt Curve/Dissociation on QuantStudio™ 3:

- 1.1. To amplify the samples and obtain the melt curve information, follow the procedure outline below.
  - 1.1.1. Open the QuantStudio™ v1.5.1 software.
    - 1.1.1.1. Create a new experiment > From Template (see **Figures 2 & 3**).
      - 1.1.1.1.1. Alternatively, select FILE, NEW EXPERIMENT, FROM TEMPLATE then browse to the “Bloodhound™ QuantStudio™ 3 Template.edt” file from the POL folder (see **Figures 2 & 3**).
      - 1.1.1.1.2. This .edt file provided by Precipio has the thermal cycler profile already programmed under Experimental Method. See **Figure 4** for a screenshot of the entire thermal cycler program.
        - 1.1.1.1.2.1. Do not change or alter any of the parameters of the thermal cycler program.
      - 1.1.1.1.3. See **Table 2** for the thermal cycler protocol.
    - 1.1.1.2. Input the experiment name under the properties tab.
      - 1.1.1.2.1. Recommended to format as DD\_MM\_YY\_PlateBarcode\_tech initials
      - 1.1.1.2.2. Confirm the following run options:
        - 1.1.1.2.2.1. Instrument type: QuantStudio™ 3 System
        - 1.1.1.2.2.2. Block type: 96-Well 0.1-mL Block
        - 1.1.1.2.2.3. Experiment type: Standard Curve
        - 1.1.1.2.2.4. Chemistry: Other
        - 1.1.1.2.2.5. Run mode: Standard
    - 1.1.1.3. Click **Plate** in the **top row to open Assign Targets and Samples** tab.
    - 1.1.1.4. Select **File -> Import Plate Setup...** to import the HRM Plate Layout Excel file made in earlier step (see **Figure 5**). The samples, plate layout, and target(s) are now assigned.
    - 1.1.1.5. Confirm all sample wells have been assigned a sample name and target. If there are empty wells, you can confirm there is no sample name or Target assigned to the well.
      - 1.1.1.5.1. Click Advanced Setup. In the 96-well Plate Layout view, select the wells you would like to exclude from analysis and then uncheck the sample name and target from the advanced setup pane on the left side.
    - 1.1.1.6. Touch  to eject the instrument drawer.
      - 1.1.1.6.1. Place the plate in the drawer so well A1 of the plate is in the top left corner.

**NOTE:** Before clicking start run, make sure your instrument is connected to your laptop. You should see your instrument serial number in the drop-down menu.

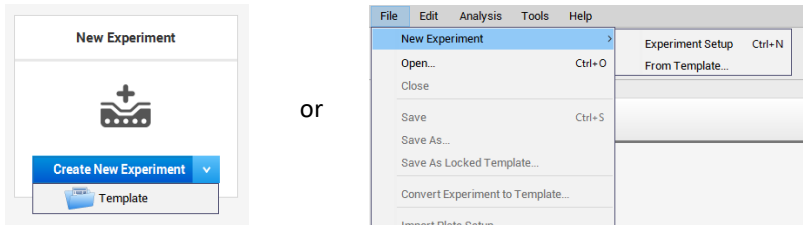
## Instructions for Use

- 1.1.1.7. Click on **START RUN** followed by the specific instrument serial number to start the run (see **Figure 6**).
- 1.1.1.8. The software will ask you to save the run. Please save the run in your designated HemeScreen® POL folder (i.e. \Egnyte\shared\HemeScreen POL\YOUR SITE).
- 1.1.1.9. The run automatically starts after saving the file.

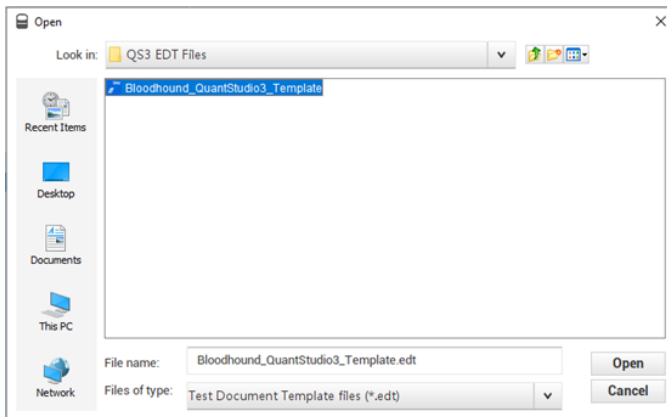
**NOTE:** QuantStudio™ automatically saves the run file on the instrument, if you failed to save the run on the computer you can always retrieve .eds file from the QuantStudio™.

**TIP:** At this stage PCR/HRM products can be stored at less than or equal to -20 °C for up to one week.

**Figure 2: Create a new experiment from Template.**



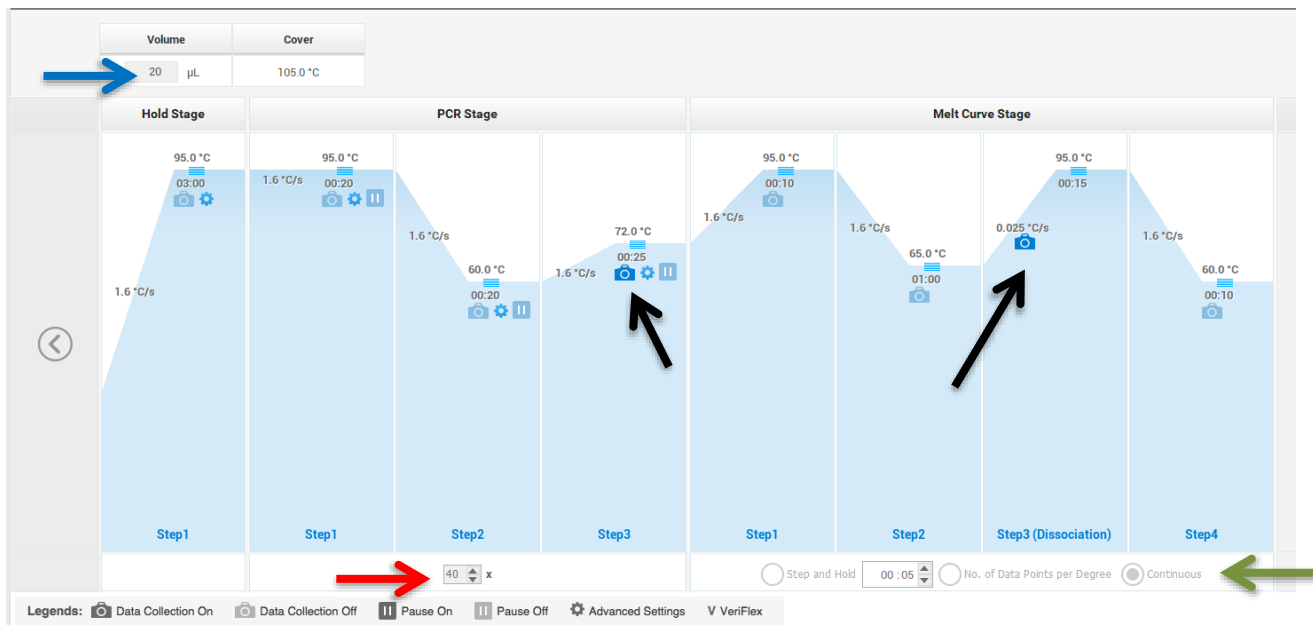
**Figure 3: Bloodhound™ QuantStudio™ Template.**



## Instructions for Use

**Figure 4: HRM thermal cycler conditions.**

Make sure reaction volume is 20  $\mu\text{L}$  (blue arrow), data collection points are selected in step 2 of PCR stage and step 3 of Melt curve stage (black arrows). Make sure PCR stage has 40 cycles (red arrow) entered and “continuous” is checked (green arrow).

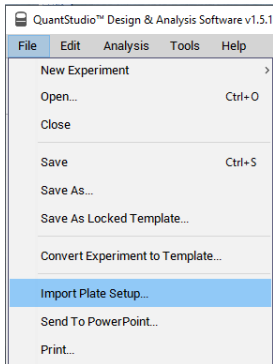


**Table 2: Thermal cycling and melt curve program for the HRM reaction.**

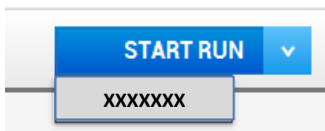
Stage	Step	Temp	Time	Ramp rate
Holding	Enzyme activation	95°C	3 min	1.6 °C/Sec
	Denature	95°C	20 sec	1.6 °C/Sec
Cycling (40 cycles)	Primer Annealing	60°C	20 sec	1.6 °C/Sec
	Extension (Data Capture on)	72°C	25 sec	1.6 °C/Sec
	Denature	95°C	10 sec	1.6 °C/Sec
Melt curve/Dissociation	Anneal	65°C	1 min	1.6 °C/Sec
	High resolution melting (Data Capture on)	95°C	15 sec	0.025 °C/Sec
	Anneal	60°C	10 sec	1.6 °C/Sec

## Instructions for Use

**Figure 5: Import Plate Layout files.**



**Figure 6: Starting the run.**



## Results

Melt curve raw data are plotted as fluorescence vs. temperature. As a product melts, the fluorescence will decrease rapidly. The Derivative Plot visualizes change in fluorescents at a given temperature. Large changes in fluorescence appear as peaks and indicate the presence and melting temperature ( $T_m$ ) of a product. The amplification control will produce a single peak at  $T_m \leq 75$  °C. For negative cases, only the single peak of the control amplicon will be visible. Positive cases will exhibit the control peak and a second peak for a fusion product.

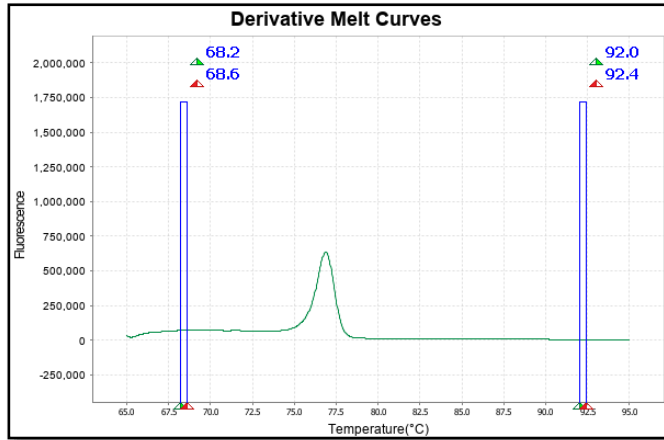
### Negative Profiles

Negative specimens will have the internal control product to ensure a successful reaction but no signal from a fusion product (**Figure 7**).

1. The amplification control melts at  $\sim 76.5$  °C.
2. If no amplification control peak is present, the reaction has failed.
3. Consider that the cDNA input or quality may be insufficient.

## Instructions for Use

**Figure 7: An example of a negative case showing the single Derivative Melt Curve peak for the internal control amplicon.**

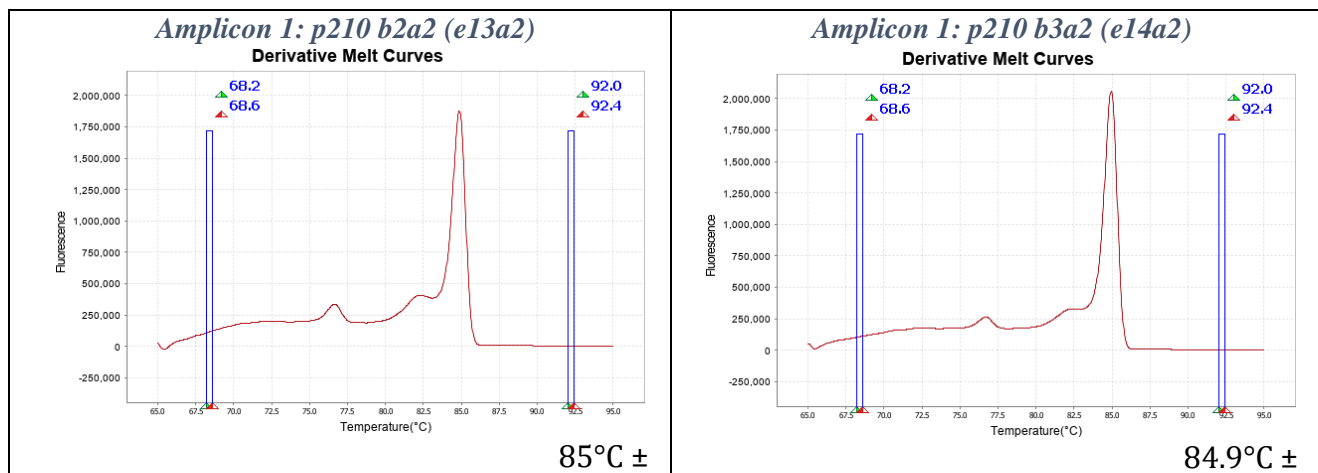


## Positive Profiles

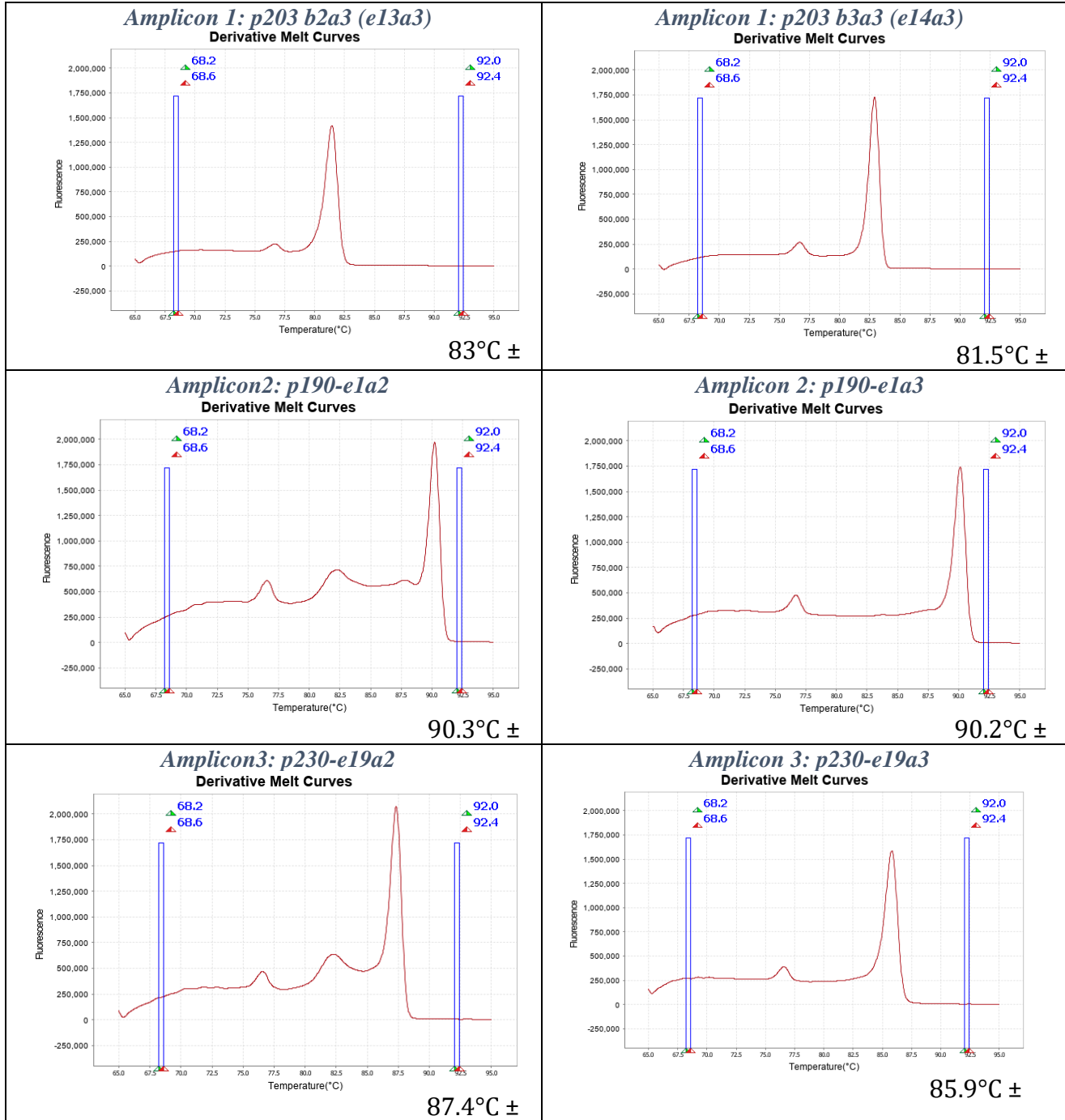
Positive specimens will have the internal control product to ensure a successful reaction and a second well defined peak with a  $T_m > 80^\circ\text{C}$  produced by the fusion product (**Figure 8**).

1. The amplification control melts at  $\sim 76.5^\circ\text{C}$  and fusion products melt at  $> 80^\circ\text{C}$
2. If no amplification control peak is present, the reaction has failed.
3. Consider that the cDNA input or quality may be insufficient. Low intensity peaks may result from co-amplification of homologous regions.

**Figure 8: Examples of positive signatures for the three assay mixes.**



Instructions for Use



## Instructions for Use

### Bloodhound Software

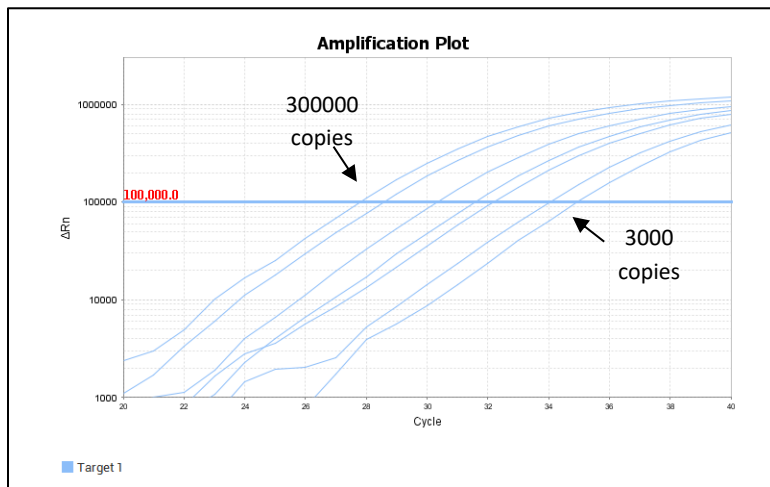
The Bloodhound Software can be accessed through this link: [Bloodhound Analysis Software](#)

**NOTE:** Refer to the Bloodhound Analysis Software User Manual for instructions on using the software.

The software utilizes the predicted  $T_m$  and the  $C_t$  values at a given threshold to determine (1) if the translocation is present and (2) relative quantification of the translocation if it is present. The analysis is based upon the instrument verification plate calibration performed. To summarize:

1. The  $C_t$  of the *ABL1* amplification from a given sample is determined and correlated to the *ABL1* copy number. If this reaction fails, the sample will fail analysis. (Figure 9).

**Figure 9: Examples of various *ABL1* copy number input from 300000 copies to 3000 copies.**



In the translocation assay mix PCRs:

1. If a single  $T_m$  is flagged for the *ABL1* internal control at  $\sim 76.5^\circ\text{C}$ , then the sample will be negative for the translocation.
2. If two  $T_m$  are present, one for the *ABL1* internal control at  $\sim 76.5^\circ\text{C}$  and one for the respective translocation at the expected  $T_m$ , the sample is positive for the translocation.

**NOTE:** The  $T_m$  will be used to delineate the p210 and p203 within the same assay mix.

3. The software will determine the threshold for the particular sample based upon the amplification curve for the *ABL1* control for the given sample.
4. The  $C_t$  value for the translocation amplification will be determined and a relative copy number will be calculated.
5. The software will then determine the estimated quantification of the translocation based upon the translocation copy number and the *ABL1* amplification.

## References

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## Warnings and Precautions

- Always wear a suitable lab coat, disposable gloves, and protective goggles when working with chemicals.
- There are no substances of animal or human origin that present a risk of infection in the supplied reagents.
- These reagents should be used only by those who have been trained in the appropriate laboratory techniques.
- Discard sample and assay waste according to your local safety regulations.

## Limitations and Disclaimers

- Bloodhound™ BCR::ABL1 is a highly sensitive technique, factors such as pipetting errors, cytosine deamination, and polymerase errors may give rise to false positives. The primers in the assay span large intronic and exonic regions and are designed to limit potential unwanted signals from contaminating genomic DNA. Co-amplification of a given sample should be taken into consideration and reported with analyzing gene expression results.
- Due to instrument-to-instrument variation, calibration variation, as well as other factors, data analysis parameters for a given data set will need to be determined by the end-user.

## Safety Information

None of the reagents present a hazard to health in the quantities supplied. The SDS form is available online as a PDF at <https://www.precipiodx.com/products/Bloodhound-reagents/>