BCR-ABL Quantitative RT PCR Kit- Major, Minor and Micro

Σ100 Rxns | -20°C

INTENDED USE

BCR-ABL Quantitative RT-PCR kit is a *in-vitro* multiplex, one-step, TaqMan probe-based quantitative assay for the specific detection and quantitation of Major, Minor and Micro BCR-ABL transcripts in the RNA/cDNA (template) extracted from EDTA whole blood or bone marrow aspirate of humans with Acute Lymphoblastic Leukemia (ALL) and Chronic Myeloid Leukemia (CML) symptoms. The kit can detect the **Deep Molecular Response up to 5 Log reduction.**

PRINCIPLE OF THE TEST

The kit uses a One-step RT-PCR master mix, which converts RNA to cDNA, followed by amplification using Taq HS DNA Polymerase. The primers and probes mix (PPMx) contains primers and probes required to amplify, detect and differentiate Major, Minor, Micro BCR-ABL transcripts in the **same reaction** along with ABL human internal control gene.

ANALYTICAL SPECIFICATION

The Quantitative standards provided in the BCR-ABL Quantitative RT PCR Kit- Major, Minor, and Micro have known copy numbers and are calibrated against WHO Ist International Genetic Reference Panel for the Quantitation of BCR-ABL translocation (09/138) for assigning IS values to a measured level of BCR-ABL/ABL %.

COMPATIBLE INSTRUMENTS

Compatible with Real-Time PCR, instruments like, BIORAD-CFX96, THERMO-QS5, QIAGEN -ROTOR-GENE Q and other instruments which supports FAM (495 nm - 520 nm), HEX (533 nm - 559 nm) Cy5 (649 nm - 670 nm) and Texas Red (589 nm - 615 nm).

PRECAUTIONS

- Treat all the specimens as potentially infectious.
- Wear protective disposable powder-free gloves, a laboratory coat and eye protection when handling specimens.
- Store positive and/or potentially positive material separated from all other components of the kit.
- Keep separate area for master mix and template preparation and work under biosafety cabinets.
- Use aerosol barrier pipette tips and frequently change the gloves.

STORAGE AND HANDLING

- Store all BCR-ABL Quantitative RT-PCR Kit reagents at -20°C.
- Do not repeatedly freeze-thaw reagents more than 5 times it leads to reduced assay sensitivity. Thaw the reagents only on ice or at 4°C.
- Kit components are stable through the end of the expiration date indicated on the box when stored at -20°C. Shelf Life is 12 Months from date of manufacturing.

MATERIALS REQUIRED BUT NOT PROVIDED

Consumables

PCR Plates/tubes

PCR plate covers/tube caps

FLUORESCENT PROBE DETAILS

Target	Reporter	Quencher
Minor BCR-ABL	FAM	BHQ1
Major BCR-ABL	HEX/VIC	BHQ2
Micro BCR-ABL	Cy5	BHQ2
ABL	TEXAS RED	BHQ2

Table 1:- Target,Reporter and Quencher

*Note: In some instruments where the BHQ option is unavailable, please set the quencher to "NONE."

KIT CONTENTS

Sl.No	Components	Volume
1.	Master Mix	2 X 625 µL
2.	Primer and Probe mix	125 µL
3.	Nuclease Free Water	1000 µL
4.	Positive Test Control	100 µL
5.	Quantitative Standard 1 (QS 1)	100 µL
6.	Quantitative Standard 2 (QS 2)	100 µL
7.	Quantitative Standard 3 (QS 3)	100 µL
8.	Quantitative Standard 4 (QS 4)	100 µL
9.	Quantitative Standard 5 (QS 5)	100 µL
10.	Quantitative Standard 6 (QS 6)	100 µL
11.	Instruction for use	1 Nos

Table 2:- Volume of kit components

REACTION MIXTURE 25 µL

Reagents	1 Rxn	20 Rxns	50 Rxns	100 Rxns
Master Mix	12.5 µL	250 µL	625 µL	1250 µL
Primer & Probe Mix	1.25 µL	25 µL	62.5 µL	125 µL
Nuclease Free Water	6.25 µL	125 µL	312.5 µL	625 µL
Total	20 µL	400 µL	1000 µL	2000 µL

Table 3:- Reaction Mixture

Use 5 µL of the test RNA (RNA samples with 50-100 ng/µL or use 10 µL of RNA for samples between 10 - 50 ng/µL and adjust the nuclease free water in the reaction mixture). For standards Add 5 µL Standards provided per reaction.

SAMPLE PREPARATION

- RNA should be extracted from human EDTA whole blood or bone marrow aspirate.
- The RNA should be extracted from human EDTA whole blood or Bone marrow aspirate.
- It is recommended to use a commercially available human Blood RNA extraction kit for the extraction. For example, NeoDx's Blood RNA extraction kit or Qiagen's RNeasy Mini Kit.

- After the extraction, the concentration of the RNA should be quantified using UV Spectrophotometer/Nanodrop/Qubit. The concentration of the RNA required for the assay should be **100-1000 ng per reaction** (Ideal and for better detection of MRD (Minimal Residual Disease).
- Evaluate the 260/280 ratio. The ratio should be closer to 2.

Note:

If the concentration of the extracted RNA is 10 ng/µL - 50 ng/µL , use 10 µL of RNA per reaction.

If the concentration of the extracted RNA is 50 ng/µL - 200 ng/µL, use 5 µL of RNA per reaction.

REACTION SET-UP

- Thaw all components of the kit on ice, mix gently using vortex and spindown the contents for 5 secs and use it immediately.
- Calculate the number of reactions for each experiment including all controls with one excess reaction volume in the reaction cocktail to accommodate pipetting errors (eg: number of reaction (n) including controls are 10 add 1 extra reaction during the preparation n+1).
- Prepare the reaction mix in a 1.5/2 mL tube for the calculated number of samples referring the table no. 3, in Master Mix Preparation room.
- Assay should be run along with positive controls and negative controls.
- Refer to table no. 3, to prepare the reaction mixture of 25 µL.
- Calculate the number of the samples to be tested along with the controls and multiply with 1 reaction volume extra to get the final reaction mixture.
- Spin down the tubes and dispense 20 µL reaction mix in each tube strips or 96 well plate.
- Before moving to template adding area, add 5 µL of nuclease free water in NTC well.

- Carefully add 5 µL RNA (or 10 µL) samples kept on ice in the designated wells in template addition room.
- Add 5 µL of Standards (QS) in the designated wells.
- Add 2 µL of PTC in a separate hood and make up the volume by adding 3 µL of nuclease free water.
- Seal the plate carefully, briefly spin down and use any qRT-PCR instrument which complies with the dyes specified in the kit insert.

THERMAL CYCLING CONDITION

STEPS	TEMP (° C)	TIME	QUANTITATION	CYCLE
Reverse transcriptase	45	20 min	Off	1
	95	2 min	Off	1
PCR and Quantitation	95	15 sec	Off	40
	60	35 sec	On	

Table 4:- Thermal Cycling Condition

STANDARD CURVE CONTROL PARAMETER

Control Parameters	Valid Value For Each Target
Slope	-3.00 to 3.74
PCR Efficiency	85%-115%
R square (R^2)	0.90-1.00

Table 5:- Curve Control Parameter

RESULT INTERPRETATION

BCR-ABL Major (HEX)	BCR-ABL Minor (FAM)	BCR-ABL Micro (Cy5)	ABL (Texas Red/ROX)	Results Validity
+	–	–	+	Valid Result
–	+	–	+	Valid Result
–	–	+	+	Valid Result
–	–	–	+	Valid Result
–	–	–	–	In valid Result

Table 6:- Assay result and interpretation

DATA ANALYSIS

- The standards should be assigned with the respective copy numbers.
- After completion of the run, analyze the data as per the RT-PCR manufacturer instructions and export the run file.
- Analysis should be performed separately for each target using a manual threshold settings..
- In case internal control has not worked for a sample re-do the test with 2 or more dilutions.

Calculations of NCN%

The copy number (starting quantity (sq)) of BCR-ABL Major, Minor, Micro and ABL should be exported to the excel sheet.

Calculate NCN% separately for each Translocation For Major NCN%.

$$\text{BCR-ABL Major NCN\%} = \frac{\text{BCR-ABL Major CN}}{\text{ABL CN}} \times 100$$

Calculations of IS %

For BCR-ABL Major IS% use the provided conversion factor along with the kit (COA or kit inside label).

$$\text{BCR-ABL Major IS\%} = \text{BCR-ABL Major NCN\%} \times \text{CF}$$

COPY NO. OF EACH STANDARD PER REACTION

Standards	Copy Numbers (BCR-ABL Major Transcript)	Copy Numbers (BCR-ABL Minor Transcript)	Copy Numbers (BCR-ABL Micro Transcript)	Copy Numbers (Internal Control ABL)
QS 1	140000000	3800000	30850000	27000000
QS 2	14000000	380000	3085000	2700000
QS 3	1400000	38000	308500	270000
QS 4	140000	3800	30850	27000
QS 5	14000	380	3085	2700
QS 6	1400	38	308.5	270

Table 7:- Copy number of each Quantitative Standards

WARNING

Positive control showed no amplification

Inappropriate storage of reagents

- *Store the reagents at recommended temperature for their optimal performance.*
- *Avoid repetitive freezing and thawing.*
- *Check the expiry of reagents.*

Negative controls are positive

Causes - Cross-Contamination

- *Follow good laboratory practices to avoid contamination issues.*
- *Use a new batch of reagents and repeat the experiment.*

Abnormal plot and/or low ΔRn values in amplification curve

The baseline was set improperly (some samples have CT values lower than the baseline value)

- *Switch from manual to automatic baseline, or move the baseline stop value to a lower CT (2 cycles before the amplification curve for the sample crosses the threshold).*

An amplification signal is detected in the early cycles

Dilute the sample to increase the CT value.