BCR-ABL Qualitative RT-PCR Kit - Major, Minor, Micro

Σ 100 Reactions | -20°C

INTENDED USE

BCR-ABL Qualitative RT-PCR Kit is a multiplex, TaqMan probe based qualitative assay for the specific detection of *Major* BCR-ABL (M-BCR), *Minor* BCR-ABL (m-BCR) and *Micro* BCR-ABL (µ-BCR) transcripts in human blood or bone marrow aspirate samples. This kit reagents enables the detection of all three BCR-ABL transcripts and ABL1 transcript (internal control) in a single tube with RNA/cDNA as a template.

BACKGROUND

BCR-ABL gene detection aids in initial diagnosis of Chronic myeloid leukemia (CML). A reciprocal translocation occurs between chromosomes 9 and 22 (t(9;22)(q34;q11)) resulting in the expression of abnormal BCR-ABL fusion tyrosine kinase. The breakpoint on chromosome 22 occurs between exons 12 and 16 of the BCR gene while the breakpoint on chromosome 9 mostly occurs between exons 1 and 2 of the ABL gene. Altogether, there are three breakpoint cluster regions in the BCR gene detected in CML patients to date: major (M-BCR), minor (m-BCR) and micro (µ-BCR). The major transcripts are called b2a2 and b3a2, which encode for a constitutively active chimeric tyrosine kinase of 210 kDa protein ($P210^{BCR\ ABL}$); minor transcript e1a2 encodes $p190^{BCR\ ABL}$ and micro transcript e19a2 encodes for $p230^{BCR\ ABL}$

PRODUCT DESCRIPTION

BCR-ABL Qualitative RT PCR kit provides reagents for screening and specific detection of Major (M-BCR) p210 ,Minor (m-BCR) p190 and Micro p230 (µ-BCR) transcripts in CML patient RNA samples. This kit is intended for "Professional Use only".

ANALYTICAL SPECIFICATION

The specificity of the kit is 100% with 100% sensitivity. The linear limit of detection of the kit for BCR-ABL Major transcript is 14 copies per µL, BCR-ABL Minor transcript is 3.8 copies per µL, BCR-ABL Micro transcript is 30.85 copies per µL and Internal control ABL is 2.7 copies per µL.

MATERIALS PROVIDED

2X Master Mix (MM) 2 * 625 µL | 20X Primer & Probe Mix (PPMx) 125 µL | Nuclease Free Water (NFW) 1000 µL | Positive Test Control (PTC) 100 µL | Instruction for use (1 No)

MATERIALS PROVIDED

KIT COMPONENTS	VOLUME
2X Master Mix (MM)	2*625 µL
20X Primer and Probe Mix (PPMx)	125 µL
Nuclease Free Water (NFW)	1000 µL
Positive Test Control (PTC)	100 µL

TARGET	REPORTER	QUENCHER
Minor BCR-ABL Transcript	FAM	BHQ1
Major BCR-ABL Transcript	HEX/VIC	BHQ2
Micro BCR-ABL Transcript	Cy5	BHQ2
ABL (Control)	Texas Red	BHQ2

*Select the quencher settings as BHQ/None

STORAGE AND HANDLING

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

PRECAUTIONS

- This product is recommended to be used by the trained professional under real-time PCR and *in-vitro* diagnostics procedure
- Treat all the specimen/sample as potentially infectious.
- Wear protective disposable powder-free gloves, a laboratory coat and eye protection when handling specimens.
- Store all the collected human samples away from the kit components before use to avoid contamination.
- Handle master mix and template preparation separately and work under biosafety cabinets.
- Use aerosol barrier pipette tips and frequently change the gloves.
- Do not open the reaction tubes/plates post-amplification, to avoid contamination with amplicons.
- Do not smoke, drink or eat in areas where kit reagents and/or human specimens are being used.
- Do not use kit components that have passed their expiration date.

WASTE DISPOSAL

- Dispose all the waste/remains of the reagents used in reaction mixture preparation and expired kit components along with bio-waste as per the lab manual/general bio-waste management instruction
- Dispose the PCR plates with patient samples "sealed" post run to avoid potential infection to the operators and contamination of the lab.

REACTION MIXTURE - 25 µL

Reagents	1 Rxn	25 Rxn	50 Rxn	100 Rxn
2X Master Mix	12.5 µL	312.5 µL	625 µL	1250 µL
20X Primer and Probe Mix	1.25 µL	31.25 µL	62.5 µL	125 µL
Nuclease Free Water	3.25 µL	81.25 µL	162.5 µL	325 µL
Total	17 µL	425 µL	850 µL	1700 µL

Add 8 µL of the test RNA per reaction

SAMPLE PREPARATION

RNA should be extracted from freshly collected EDTA whole blood or bone marrow aspirates using any approved RNA extraction methods. Store the extracted RNA at - 20 °C for further use.

TEST PREPARATION/REACTION SET-UP

1. Thaw all components of the kit on ice, mix gently using vortex and spindown the contents for 5 secs and use it immediately.
2. 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)
3. Prepare the reaction mix in a 1.5/2 mL tube for the calculated number of samples in Master Mix Preparation room.
4. Spin down the tubes and dispense 17 µL reaction mix in each tube strips or 96 well plate. Before moving to template adding area, add 8 µL of nuclease free water in NTC wells.
5. Carefully add 8 µL samples kept on ice in the designated wells in template addition room. Add 2 µL of PTC in a separate hood and make up the volume by adding 6 uL of nuclease free water. The assay should be run along with positive controls and negative controls.

THERMAL CYCLIC CONDITIONS

STEP	TEMP °C	TIME	DETECTION	CYCLE
Reverse Transcription	45 95	20 min 2 min	Off	1
PCR and Detection	95	15 Sec	Off	40
	60	35 Sec	On	

READING TEST RESULTS / DATA ANALYSIS

- NTCs should be negative and should not exhibit fluorescence amplified curves that cross the threshold line.
- If a false positive occurs with one or more of the primer and probe in NTC reactions , it indicates sample contamination.
- In that case, Invalidate the run and repeat the assay with stricter adherence to the procedure guidelines.
- Positive control should produce a positive result with an expected Ct value for each target included in the test.
- If expected positive reactivity is not achieved, invalidate the run and repeat the assay with stricter adherence to procedure guidelines.
- After completion of the run, analyze the data as per the instrument manufacturer instructions.
- 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.

RESULTS INTERPRETATION

Major BCR -ABL,Minor BCR-ABL,Micro BCR-ABL	ABL (Internal Control)	Assay result
Ct < 40	Ct ≤ 40	Positive
Ct = Undetermined or Ct ≥ 40	Ct ≤ 40	Negative
Ct = Undetermined or Ct ≥ 40	Ct ≥ 40 Undetermined	Invalid. Re-purify the nucleic acid from the sample, then repeat the test.

DATA INTERPRETATION

Minor BCR-ABL (mBCR-ABL) FAM	Major BCR-ABL (MBCR-ABL) HEX/VIC	Micro BCR-ABL (µ-BCR-ABL) Cy5	ABL Texas Red	RESULTS INTERPRETATION
+	-	-	+	Minor BCR-ABL Transcript detected
-	+	-	+	Major BCR-ABL Transcript detected
-	-	+	+	Micro BCR-ABL Transcript detected
-	-	-	+	No fusion transcripts of BCR-ABL detected
-	-	-	-	Invalid run. Repeat the extraction and re-run

TROUBLESHOOTING

Positive control showed no amplification

Inappropriate storage of reagents

- Store the reagents at recommended temperature for their optimal performance.
- Avoid freeze-thaw more than the recommended number of times
- 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