PML-RARA Qualitative Real-Time PCR Kit

INTENDED USE

PML-RARA Qualitative Real Time PCR kit is an *in-vitro* multiplex kit for the detection of PML-RARA bcr1 (long) ,bcr2 (variant) and bcr3 (short) transcripts from RNA extracted from peripheral blood or bone marrow aspirates , along with the internal control ABL1 in a single tube assay to diagnose Acute Promyelocytic Leukemia.

SAMPLE TYPE

EDTA whole blood or bone marrow aspirates

BACKGROUND

Promyelocytic leukemia, also known as acute promyelocytic leukemia (APL), is a subtype of acute myeloid leukemia (AML). It is characterized by an abnormal fusion gene sequence called PML-RARA, resulting from a reciprocal translocation between chromosome 15 and chromosome 17, commonly represented as t(15;17). This fusion occurs when segments of these chromosomes break off and switch places (translocate). Specifically, the PML gene region on chromosome 15 fuses with the RARA gene region on chromosome 17. To diagnose APL, a test is performed to detect the presence of the PML-RARA fusion gene in the blood or bone marrow of an individual.

PRODUCT DESCRIPTION

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 and detect bcr1,bcr2 and bcr3 in the same reaction along with ABL human internal control gene.

PRINCIPLE

PML-RARA Qualitative Real Time PCR Kit is an *in-vitro* multiplex assay for the detection of bcr1,bcr2 & bcr3 transcripts from peripheral blood or bone marrow aspirate.This is a multiplex assay kit where primer and probe sets are designed to target the PML-RARA transcripts - bcr1,bcr2and bcr3.

MATERIALS PROVIDED

KIT COMPONENTS	VOLUME
Master Mix	2x625 µL
Primer and Probe mix	125 µL
Nuclease Free Water	1000 µL
Positive Test Control	100 µL
Instruction for use	1 No.

MATERIALS REQUIRED BUT NOT PROVIDED

Consumables
PCR Plates/tubes
PCR plate covers/tube caps

COMPATIBLE INSTRUMENTS

Compatible with Real-Time PCR, instruments like, BIORAD-CFX96 , THERMO-QS5, QIAGEN-ROTOR-GENE Q and other instruments which supports HEX (535 nm - 556 nm) and Texas Red (596 nm - 615 nm).

TARGET	REPORTER	QUENCHER
PML-RARA bcr1,bcr2,bcr3	FAM	BHQ1
ABL1	Texas Red/ROX	BHQ2

*Select the quencher settings as BHQ/None

STORAGE AND HANDLING

- Store all PML-RARA Qualitative Kit reagents at -20°C.
- Do not repeatedly freeze-thaw reagents more than five times as 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 - 12 Months from date of manufacturing.

PRECAUTIONS

- It is recommended that this product is used by personnel specially instructed and trained in real-time PCR and in-vitro diagnostics procedures.
- 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 areas for master mix and template preparation 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.

REACTION MIXTURE - 25 µL

Reagents	1 Rxn	20 Rxn	50 Rxn	100 Rxn
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

Add 5 µL of the test RNA/cDNA 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 ℃ for further use.

TEST PREPARATION/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 in Master Mix Preparation room.
- 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 wells.
- Carefully add 5 µL of RNA/cDNA 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 3 µL of nuclease free water. The assay should be run along with positive controls and negative controls.
- Seal the plate carefully, briefly spin down and use any qRT-PCR instrument which complies with the dyes specified in the kit insert.

THERMAL CYCLIC CONDITIONS

STEP	TEMP °C	TIME	DETECTION	CYCLE
Reverse Transcription	52	4 Min	Off	1
	95	1 Min	Off	1
PCR and Detection	95	5 Sec	Off	40
	57	40 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.
- Negative results do not exclude possibility of infection and should not be used as the sole basis for the treatment.

RESULTS INTERPRETATION

For PML-RARA bcr1,bcr2 & bcr3	For ABL1	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 and repeat the test.

DATA INTERPRETATION

PML-RARA bcr1, bcr2, bcr3	ABL-1	Results Interpretation
+	+	PML-RARA bcr1,bcr2 & bcr3 detected
-	+	Absence of PML RARA translocation
-	-	Invalid Run.Repeat RNA extraction and PCR run

WASTE DISPOSAL

- Dispose all the waste/remains of the reagents used in reaction mixture preparation & 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.

TROUBLESHOOTING

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