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# Leukemia BCR-ABL Fusion Gene (μ-BCR) Real Time RT-PCR Kit
**User Manual**

# LT066210RT50

***For use with ABI Prism®7000/7300/7500/7900/Step One Plus; iCycler iQ™4/iQ™5; Smart Cycler II;Bio-Rad CFX 96;Rotor Gene™6000; Mx3000P/3005P;MJ-Option2/ Chromo4; LightCycler®480 Instrument***

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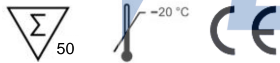



**1. Intended Use**
Leukemia BCR-ABL Fusion Gene (μ-BCR) real time RT-PCR Kit is used for the detection of micro(μ) BCR-ABL gene variants (e19a2) in leukocyte by using real time PCR systems.

**2. Principle of Real-Time PCR**
The principle of the real-time detection is based on the fluorogenic 5'nuclease assay. During the PCR reaction, the DNA polymerase cleaves the probe at the 5' end and separates the reporter dye from the quencher dye only when the probe hybridizes to the target DNA. This cleavage results in the fluorescent signal generated by the cleaved reporter dye, which is monitored real-time by the PCR detection system. The PCR cycle at which an increase in the fluorescence signal is detected initially is proportional to the amount of the specific PCR product. Monitoring the fluorescence intensities in rea-time allows the detection of the accumulating product without having to re-open the reaction tube after the amplification.

**3. Product Description**
The BCR-ABL fusion gene is associated with formation of the Philadelphia chromosome (Ph) and is one of the most common genetic abnormalities detected in leukaemia's. In the vast majority of patients, the breakpoints in the BCR gene are clustered within three well defined regions. One fusion gene called μ-bcr ,it is ver rare. The breakpoint located in the bcr region, resulting in an e19a2 fusion transcript, which encodes for a longer 230 kDa BCR-ABL protein. e19a2 CML has been associated with chronic neutrophilic leukaemia (CNL).Leukemia BCR-ABL Fusion Gene(μ-BCR) real time RT-PCR kit contains a specific ready-to-use system for the detection of the Leukemia BCR-ABL Fusion Gene(μ-BCR) using RT-PCR (Reverse Transcription Polymerase Chain Reaction) in the real-time PCR system. The master contains Super Mix for the specific amplification of μ-BCR. The reaction is done in one step real time RT-PCR. The first step is a reverse transcription (RT), during which the Leukemia BCR-ABL Fusion Gene(μ-BCR) is transcribed into cDNA. Afterwards, a thermostable DNA polymerase is used to amplify the specific gene fragments by means of PCR (polymerase chain reaction). Fluorescence is emitted and measured by the real time systems optical unit during the PCR. The detection of amplified BCR-ABL fragment is performed in fluorimeter **channel FAM**.

**4. Kit Contents**

| Ref. | Type of reagent | Presentation | 50rxns |
|---|---|---|---|
| 1 | μ-BCR Super Mix | 1 vial, 480μl | x2 |
| 2 | RT-PCR Enzyme Mix | 1 vial, 28μl | x2 |
| 3 | Molecular Grade Water | 1 vial, 400μl | x2 |
| 4 | μ-BCR Positive Control($1\times10^7$copies/ml) | 1 vial, 30μl | x2 |

**Analysis sensitivity: $1\times10^4$copies/ml**
**Note:** Analysis sensitivity depends on the sample volume, elution volume, nucleic acid extraction methods and other factors .If you use the RNA extraction kits recommended, the analysis sensitivity is the same as it declares. However, when the sample volume is dozens or even hundreds of times greater than elution volume by some concentrating method, it can be much higher.

**5. Storage**
- All reagents should be stored at **-20℃**. Storage at +4℃ is not recommended.
- All reagents can be used until the expiration date indicated on the kit label.
- Repeated thawing and freezing (> 3x) should be avoided, as this may reduce the sensitivity of the assay.
- Cool all reagents during the working steps.
- Super Mix should be stored in the dark.

**6. Additionally Required Materials and Devices**
- Biological cabinet
- Real time PCR system
- Desktop microcentrifuge for "eppendorf" type tubes (RCF max. 16,000 x g)
- Vortex mixer
- RNA extraction kit
- Real time PCR reaction tubes/plates
- Cryo-container
- Pipets (0.5 μl – 1000 μl)
- Sterile filter tips for micro pipets
- Sterile microtubes
- Disposable gloves, powderless
- Biohazard waste container
- Refrigerator and freezer
- Tube racks

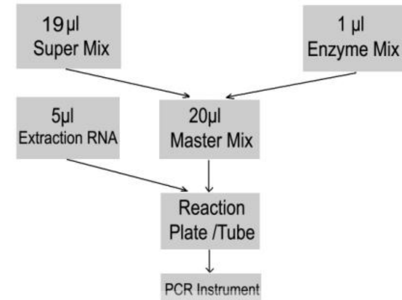
**7. ⚠ Warnings and Precaution**
- Carefully read this instruction before starting the procedure.
- For in vitro diagnostic use only.
- This assay needs to be carried out by skilled personnel.
- Clinical samples should be regarded as potentially infectious materials and should be prepared in a laminar flow hood.
- This assay needs to be run according to Good Laboratory Practice.
- Do not use the kit after its expiration date.
- Avoid repeated thawing and freezing of the reagents, this may reduce the sensitivity of the test.
- Once the reagents have been thawed, vortex and centrifuge briefly the tubes before use.
- Prepare quickly the Reaction mix on ice or in the cooling block.
- Set up two separate working areas: 1) Isolation of the RNA/ DNA and 2) Amplification/ detection of amplification products.
- Pipets, vials and other working materials should not circulate among working units.
- Use always sterile pipette tips with filters.
- Wear separate coats and gloves in each area.
- Do not pipette by mouth. Do not eat, drink, smoke in laboratory.
- Avoid aerosols.

**8. Sample Collection, Storage and transport**
- Collected samples in sterile tubes.
- Specimens can be extracted immediately or frozen at -20℃ to -80℃.
- Transportation of clinical specimens must comply with local regulations for the transport of etiologic agents.

**9. Procedure**
**9.1 RNA-Extraction**
RNA extraction kits are available from various manufacturers. You may use your own extraction systems or the commercial kit based on the yield. For the RNA extraction, please comply with the manufacturer's instructions. The recommended extraction kit is as follows:

| Nucleic Acid Isolation Kit | Cat. Number | Manufacturer |
|---|---|---|
| RNA Isolation Kit | EM-0100/EM-2100 | Life Tech |
| QIAamp Viral RNA Mini extraction Kit (50) | 52904 | QIAGEN |

**9.2 RT-PCR Protocol**
The Master Mix volume for each reaction should be pipetted as follows:




1) The volumes of Super Mix and Enzyme Mix per reaction multiply with the number of samples, which includes the number of controls and sample prepared. Molecular Grade Water is used as the negative control. For reasons of unprecise pipetting, always add an extra virtual sample. Mix completely then spin down briefly in a centrifuge.
2) Pipet **20μl** μ-BCR Master Mix with micropipets of sterile filter tips to each of the real time PCR reaction plate/tubes. Separately add **5μl** RNA sample, positive and negative controls to different plate/tubes. Immediately close the plate/tubes to avoid contamination.
3) Spin down briefly in order to collect the Master Mix in the bottom of the reaction tubes.
4) Perform the following protocol in the instrument:

| | |
|---|---|
| 45℃ for 10min | 1cycle |
| 95℃ for 15min | 1cycle |
| 95℃ for 15sec, 60℃ for 1min ( Fluorescence measured at 60℃) | 40cycles |

| Selection of fluorescence channels | |
|---|---|
| FAM | Target Nucleic Acid |
| HEX/VIC/JOE | IC |

5) ⚠ If you use ABI Prism® system, please choose **"none"** as **passive reference** and **quencher.**

**10. Threshold setting:** just above the maximum level of molecular grade water.

**11. Quality control:**
Negative control, positive control and internal control must be performed correctly, otherwise the sample results is invalid.

| Channel | Ct value |
|---|---|
| Control | FAM |
| Molecular Grade Water | UNDET |
| Positive Control(qualitative assay) | ≤35 |

**12. Data Analysis and Interpretation**
The following results are possible:

| | Ct value | Result Analysis |
|---|---|---|
| 1# | UNDET | Below the detection limit or negative |
| 2# | ≤38 | The sample contains micro(μ) BCR-ABL gene variants (e19a2), and the software displays the quantitative value |
| 3# | 38～40 | Re-test; if it is still 38~40, report as 1# |

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