Leukemia BCR-ABL Fusion Gene

Real Time RT-PCR Kit

Cat. No.: TR-0126-01

For Use with LightCycler 1.0/LightCycler2.0/LightCycler480 (Roche) Real Time PCR Systems

For In Vitro Diagnostic Use Only

User Manual

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1. Intended Use
Leukemia BCR-ABL Fusion Gene real time RT-PCR Kit is used for the detection of Leukemia BCR-ABL Fusion Gene (M-BCR, m-BCR, and μ-BCR) in leukocyte by using real time PCR systems.

2. Introduction
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 involves breaks within the major breakpoint cluster region (M-BCR) of BCR, which includes introns 13 and 14, and leads to the production of an 8.5 kb transcript coding for a 210 kDa protein (p210). In CML, most translocations involve the M-BCR of BCR.

3. 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 (Ct) is proportional to the amount of the specific PCR product. Monitoring the fluorescence intensities during Real Time allows the detection of the accumulating product without having to re-open the reaction tube after the amplification.

4. Product Description
Leukemia BCR-ABL Fusion Gene real time RT-PCR kit contains a specific ready-to-use system for the detection of the Leukemia BCR-ABL Fusion Gene using RT-PCR (Reverse Transcription Polymerase Chain Reaction) in the real-time PCR system. The master contains three Super Mixes for the specific amplification of M-BCR, m-BCR and 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 (M-BCR, m-BCR, 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 with the fluorescent quencher BHQ1. An external positive control (1×10⁷ copies/ml) supplied, allows the determination of the gene load. For further information, please refer to section 10.2 Quantitation.

5. Kit Contents

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Type of reagent</th>
<th>Presentation 25rxns</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M-BCR Super Mix</td>
<td>1 vial, 380µl</td>
</tr>
<tr>
<td>2</td>
<td>m-BCR Super Mix</td>
<td>1 vial, 380µl</td>
</tr>
<tr>
<td>3</td>
<td>μ-BCR Super Mix</td>
<td>1 vial, 380µl</td>
</tr>
<tr>
<td>4</td>
<td>RT-PCR Enzyme Mix</td>
<td>1 vial, 84µl</td>
</tr>
<tr>
<td>5</td>
<td>Molecular Grade Water</td>
<td>1 vial, 400µl</td>
</tr>
<tr>
<td>6</td>
<td>BCR-ABL Positive Control (1×10⁷ copies/ml)</td>
<td>1 vial, 90µl</td>
</tr>
</tbody>
</table>

6. Storage
- All reagents should be stored at -20°C. Storage at +4°C 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.

7. 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

8. 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

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

10. Procedure
10.1 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::
### 10.2 Quantitation

The kit can be used for **quantitative** or **qualitative** real-time RT-PCR. A positive control defined as $1 \times 10^7$ copies/ml is supplied in the kit.

**For performance of quantitative real-time PCR, Standard dilutions must prepare first as follows.**

**Molecular Grade Water is used for dilution.**

**Dilution is not needed for performance of qualitative real-time PCR.**

Take positive control ($1 \times 10^7$ copies/ml) as the starting high standard in the first tube. Respectively pipette 36ul of Molecular Grade Water into next three tubes. Do three dilutions as the following figures:

![Dilution of Standards](image)

To generate a standard curve on the real-time system, all four dilution standards should be used and defined as standard with specification of the corresponding concentrations.

**Attention:**

A. Mix thoroughly before next transfer.

B. The positive control ($1 \times 10^7$ copies/ml) contains high concentration of the target DNA. Therefore, be careful during the dilution in order to avoid contamination.

### 10.3 RT-PCR Protocol

The Master Mix volume for each reaction should be pipetted as follows:
1) Depending upon the number of samples(n) the following pipetting scheme can be followed. (For reasons of unprecise pipetting, always add an extra virtual sample.)

<table>
<thead>
<tr>
<th>Reaction Volume</th>
<th>Master Mix Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>14µl Super Mix</td>
<td>14µl × (n+1)</td>
</tr>
<tr>
<td>1µl Enzyme Mix</td>
<td>1µl × (n+1)</td>
</tr>
</tbody>
</table>

Mix completely then spin down briefly in a centrifuge.

2) Pipet 15µl M-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) Pipet 15µl m-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.

4) Pipet 15µl 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.

5) Spin down briefly in order to collect the Master Mix in the bottom of the reaction tubes.

6) Perform the following protocol in the instrument:

   45°C for 10 min, 1 cycle;

   95°C for 15 min, 1 cycle;
11. Data Analysis and Interpretation

The following results are possible:

1) A signal is detected in channel FAM of M-BCR Super Mix. **The result is positive: The sample contains major BCR-ABL gene variants (e13a2 and e14a2),**

2) A signal is detected in channel FAM of m-BCR Super Mix. **The result is positive: The sample contains minor BCR-ABL gene variants (e1a2).**

3) A signal is detected in channel FAM of μ-BCR Super Mix. **The result is positive: The sample contains micro(μ) BCR-ABL gene variants (e19a2).**

4) In channel FAM no signal is detected of M-BCR Super Mix, m-BCR Super Mix or μ-BCR Super Mix. **The sample does not contain Leukemia BCR-ABL Fusion Gene. It can be considered negative.**

For further questions or problems, please contact our technical support at trade@liferiver.com.cn