1. Intended Use
Leukemia BCR-ABL Fusion Gene (m-BCR) Real Time RT-PCR Kit is used for the detection of minor BCR-ABL gene variants (e1a2) 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 Ct value is the cycle at which an increase in the fluorescent signal is detected initially. The Ct value is proportional to the amount of the specific PCR product. Monitoring the fluorescence intensities in real-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. In the vast majority of patients, the breakpoints in the BCR gene are clustered within three well defined regions. One fusion gene is the result of breaks within the first intron of BCR (the minor breakpoint cluster region, m-bcr) and leads to the expression of a smaller 7.5 kb transcript that codes for a 1 kDa protein, the BCR-ABL fusion protein. The ABL gene is located at 9q34 and is associated with the formation of the Ph (Philadelphia chromosome) and is one of the most common genetic abnormalities detected in leukaemia.

4. Kit Contents

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. m-BCR Super Mix</td>
<td>1 vial</td>
</tr>
<tr>
<td>2. RT-PCR Enzyme Mix</td>
<td>1 vial</td>
</tr>
<tr>
<td>3. Molar Grade Water</td>
<td>1 vial</td>
</tr>
<tr>
<td>4. m-BCR Prepositive Control (1×10 copies/ml)</td>
<td>1 vial</td>
</tr>
</tbody>
</table>

5. 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.

6. Additional Materials and Devices
- Biological cabinet
- Vortex mixer
- Cryo-container
- Sterile filter tips for micro pipets
- Disposable gloves, powder-free
- Biohazard waste container
- Refrigerator and Freezer
- Desktop microcentrifuge for "eppendorf" type tubes (RCF max. 16,000 x g)

7. Precautions and Warnings
- Carefully read this instruction before starting the procedure.
- In vitro diagnostic use only.
- This assay cannot be carried out by skilled personnel.
- Clinical samples should be regarded as potentially infectious materials and should be prepared in a laminar flow hood.
- Use only 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°C to -80°C.
- 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:

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Cat. Number</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZJ Biotech</td>
<td>ME-0010/ME-0012</td>
<td>QIAamp Viral RNA Mini extraction Kit</td>
</tr>
</tbody>
</table>

9.2 Quantitation
The kit can be used for quantitative or qualitative real-time RT-PCR. A positive control defined as 1×10 copies/ml is supplied in the kit.

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

11. Calibration for quantitative detection: Input each concentration of standard controls at the end of run, and a standard curve will be automatically formed.

12. Quality control:
Negligible control, positive control and CS curve must be performed correctly, otherwise the sample results is invalid.

13. Data Analysis and Interpretation
The following results are possible:

<table>
<thead>
<tr>
<th>Threshold</th>
<th>Result Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤38</td>
<td>REtest; if it is still ≤38, report as ≤38</td>
</tr>
<tr>
<td>&gt;38&lt;58</td>
<td>UNDETe</td>
</tr>
<tr>
<td>&gt;58</td>
<td>Below the detection limit or negative</td>
</tr>
</tbody>
</table>

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