Mycobacterium tuberculosis (TB) PCR Fluorescence Quantitative Diagnostic Kit

Service manual
(48T)

For research use only
Introduction

Mycobacterium Tuberculosis (TB) is still one of the most important infectious diseases worldwide. Some two billion people, one-third of the world's population, are infected with Mycobacterium tuberculosis. Tuberculosis, a chronic, cyclic disease, mainly affecting the lung and the associated lymph nodes. The kit adopts one pair of primers and a particularly designed probes, using PCR amplification and in vitro internal standard technology to detect Mycobacterium tuberculosis, which could assist the diagnosis and prognosis of mycobacterium tuberculosis infection.

Ingredients

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Volume</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleic acid extraction solution</td>
<td>1.2ml</td>
<td>2 Tube(s)</td>
</tr>
<tr>
<td>TB PCR reaction buffer</td>
<td>840ul</td>
<td>2 Tube(s)</td>
</tr>
<tr>
<td>Taq polymerase</td>
<td>19.2ul</td>
<td>1 Tube(s)</td>
</tr>
<tr>
<td>Critical positive control</td>
<td>200ul</td>
<td>1 Tube(s)</td>
</tr>
<tr>
<td>Negative control</td>
<td>200ul</td>
<td>1 Tube(s)</td>
</tr>
<tr>
<td>Positive control</td>
<td>200ul</td>
<td>1 Tube(s)</td>
</tr>
<tr>
<td>Internal control</td>
<td>48ul</td>
<td>1 Tube(s)</td>
</tr>
<tr>
<td>Reference 1: (1~5)×10^7 copies/ml</td>
<td>40ul</td>
<td>1 Tube(s)</td>
</tr>
<tr>
<td>Reference 2: (1~5)×10^6 copies/ml</td>
<td>40ul</td>
<td>1 Tube(s)</td>
</tr>
<tr>
<td>Reference 3: (1~5)×10^5 copies/ml</td>
<td>40ul</td>
<td>1 Tube(s)</td>
</tr>
<tr>
<td>Reference 4: (1~5)×10^4 copies/ml</td>
<td>40ul</td>
<td>1 Tube(s)</td>
</tr>
</tbody>
</table>

Applied instrument

Line-Gene (II) or Line-Gene K Real-time PCR detection system.

Sample collection, storage and transport

Take 1~3ml the first deep sputum in the morning with sterile 5 ml glass tube, or take Cerebrospinal fluid, Pleural effusion, Ascites and the phlegm of other suspected TB infected position and seal it as soon as possible. The sample can be used immediately or store at -20 °C for inspection. Please avoid repeating frozen and transport the sample at or lower than 0°C.

Please store the sample at -20°C. please refer This kit will be valid for one year when stored at -20°C 〈please use the kit in the period of validity〉

Protocol

1 Specimen preparation

Add four times volume of 4% NaOH,vortex, hold 30 minutes at RT for liquefaction. Take 1ml liquid and transfer into a 2 ml centrifuge tube. Add 1ml 4% NaOH, stay 10 minutes at RT. Centrifuge at 14,000rpm for 5 minute and discard the upper supernatant. Add 1 ml sterile saline and mix thoroughly. Centrifuge 14,000rpm for 5 minutes, and discard the upper supernatant. Add 50ul nucleic acid extraction solution, mix thoroughly. Hold 10 minutes at 100°C, then turn back to RT. Centrifuge 10,000 rpm for 1 minute. Take 4 ul supernatant for
next PCR reaction.

For cerebrospinal fluid, Pleural effusion, Ascites specimen, take 1 ml liquid to 1.5ml centrifuge tube. Discard the upper supernatant. Add 50ul nucleic acid extraction solution, mix thoroughly. Hold 10 minutes at 100℃, then turn back to RT. Centrifuge 10,000 rpm for 1 minute. Take 4 ul supernatant for next PCR reaction.

2 **Standard quality control preparation**

Positive and negative control of the alternative reference turn to RT, 14,000rpm for 1 minute, and discard the upper supernatant. Add 50ul nucleic acid extraction solution, mix thoroughly. Hold 10 minutes at 100℃, then turn back to RT. Centrifuge 10,000 rpm for 1 minute. Take 4 ul supernatant for next PCR reaction.

3 **Reagent preparation**

Defrosting the reagents(PCR reaction solution, Taq polymerase ),references and controls in the kit at room temperature. Before preparing PCR reagents, please centrifuge all reagents for a few seconds. Make PCR reagents according to the quantity of sample, controls and references as below:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>PCR reagent</th>
<th>Taq polymerase</th>
<th>Internal control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dosage</td>
<td>34.6 ul</td>
<td>0.4 ul</td>
<td>1ul</td>
</tr>
</tbody>
</table>

After mixing PCR reagents above, distribute them into 0.2ml PCR tubes as 36ul per tube. After placed in the refrigerator at -20℃, storage time should not exceed 24 hours.

4 **Adding sample**

Add nucleic acid sample, controls and references as per 4ul each into PCR tubes above. 8000 rpm centrifuge for 10 sec.

5 **PCR reaction**

Setting reaction procedure as following:

94℃: 5 min;
94℃: 15 sec
60℃: 15 sec
72℃: 40 sec

40 cycles

6 **Select the channel of instrument for testing**

Choose F1（FAM）and F2(HEX) channels when collecting fluorescent signals and set fluorescent signals detecting at 72℃.

*Before running, please adjust gain value to make the F1（FAM） background between 15-25 and F2（HEX） background between 20-30.*

**Result analysis and judgments**

- Input the concentrations of four References. Select fit point method to analyze.Confirm the base line (zero adjustment) by getting the fluorescent signals of 12-14 cycles for F1（FAM） and 23-25 for F2（HEX）. Make the noise limit just beyond the peak of the amplification curve (ruleless noise line) of normal negative control; then do quantitative analysis. You could also adjust by yourself according to the condition of instrument’s noises.

- If the result of $1 \times 10^8$ copies/ml $\geq$ TB DNA $\geq$ $1 \times 10^3$ copies/ml, the result is available, and you can report relative copies directly.
If TB DNA $\geq 1 \times 10^8$ copies/ml in the test sample, you can report as $\geq 1 \times 10^8$ copies/ml directly, or you can use sterile saline to dilute as per 10 times of grads, then re-test when the copies is among $1 \times 10^5 \sim 5 \times 10^7$ copies/ml, adjusting test result by dilution times.

When the test sample's $1 \times 10^3 \geq$TB DNA $\geq 1 \times 10^2$ copies/ml, make a double re-test and if the result of double re-test still among $1 \times 10^2 \sim 1 \times 10^3$ copies/ml, report as actual value. If both or one re-trial test is lower than $1 \times 10^5$ copies/ml, report TB DNA $< 1 \times 10^5$ copies/ml.

When TB DNA $< 1 \times 10^2$ copies/ml in the test sample, report TB DNA $< 1 \times 10^2$ copies/ml.

When the test sample's $1 \times 10^3 \geq$TB DNA $< 1 \times 10^2$ copies/ml, make a double re-test and if the result of double re-test still among $1 \times 10^2 \sim 1 \times 10^3$ copies/ml, report as actual value. If both or one re-trial test is lower than $1 \times 10^5$ copies/ml, report TB DNA $< 1 \times 10^5$ copies/ml.

When the CT value is zero, report negative judgment.

**Quality Control**

- Coefficient of correlation of the standard curve $\leq -0.970$.
- Negative control, all are negative. The positive control TB DNA is among $1 \times 10^5 \sim 5 \times 10^7$ copies/ml. Critical control TB DNA is among $1 \times 10^3 \sim 9 \times 10^4$ copies/ml. Otherwise experiment deemed null and void.
- Internal control: all are positive. And Ct value is among $\leq 35$; If the Ct value is $>35$, it can be convicted that PCR determination is inhibited and re-sample dilution or re-determination is proposed.

**Important note**

1. *For research use only.* Please make sure the reagent is in period of validity.
2. Please read this manual carefully before beginning the experiment.
3. Wear disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Thoroughly wash hands afterward.
4. Do not pipette by mouth. Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
5. Dispose all specimens and unused reagents in accordance with local regulations.
6. Biosafety Level 2 should be used for materials that contain or are suspected of containing infectious agents.
7. Avoid contact of specimens and reagents with the skin, eyes and mucous membranes. If these solutions come into contact, rinse immediately with water and seek medical advice immediately.
8. Use of this product should be limited to personnel trained in the techniques of DNA amplification.
9. Workflow in the laboratory must proceed in a uni-directional manner, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where you performed previous step.

**Storage and period of validity**

Please store the kit at -20°C. This kit will be valid for one year when stored at -20°C (please use the kit in the period of validity).

**Information of Manufacturer**

HANGZHOU BIOER TECHNOLOGY CO., LTD.
Address: 1192 Bin An Rd., Hi-Tech (Binjiang) District, Hangzhou, 310053, China
Website: www.bioer.com.cn.
TEL: +86-571-87774575;
FAX: +86-571-87774565.