Mycoplasma Pneumoniae (MP) Real Time PCR Kit User Manual

For In Vitro Diagnostic Use Only

REF: RD-0100-02

For use with ABI Prism™7000/7300/7500/7800/Step One Plus; iCycler iQ®+iQ5™; Smart Cycler II/Bio-Rad CFX 96; Rotorgene® 6000; Mx3000P/3050P; MJ-Option2/Chromo4; LightCycler®480 Instrument

EC 467
Obelia S.A.
Boulevard Général Wahis 53
1030 Brussels, BELGIUM
Tel: +32 (2) 732.59.54
Fax: +32 (2) 732.60.03
E-mail: mail@obelia.net

1. Intended Use

MP real time PCR kit is used for the detection of mycoplasma pneumoniae by real time PCR systems in samples like nasal and pharyngeal secretions, sputum, provoked sputum, bronchial lavage, lung biopsy pleural effusion and etc. 

2. Principle of Real-Time PCR

The principle of the real-time detection is based on the fluorogenic 5’-nuclease assay. During 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 fluorescence 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.

3. Product Description

Mycoplasma pneumoniae is an important etiological agent of community acquired infections of upper and lower respiratory tracts in children and young adults, mainly atypical pneumonia. M. pneumoniae has been reported as the cause of 25-30 % of all pneumonia cases. This pathogen has also been associated with non respiratory diseases as meningitis, encephalitis, pancreatitis or arthritis.

Mycoplasma Pneumoniae real time PCR kit contains a specific ready-to-use system for the detection of mycoplasma pneumoniae by polymerase chain reaction (PCR) in the real-time PCR system. The kit contains reagents and enzymes for the specific amplification of MP DNA. Fluorescence is emitted and measured by the real time system optical unit during PCR. The detection of amplified MP DNA fragment is performed in fluorimeter channel FAM with the fluorochrome BHQ1. DNA extraction buffer is available in the kit. In addition, the kit contains a system to identify possible PCR inhibition by measuring the HEX/VIC/JOE fluorescence of the internal control (IC).

4. 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>DNA Extraction Buffer</td>
<td>1 vial, 1.5ml</td>
</tr>
<tr>
<td>2</td>
<td>MP Reaction Mix</td>
<td>1 vial, 950µl</td>
</tr>
<tr>
<td>3</td>
<td>PCR Enzyme Mix</td>
<td>1 vial, 12µl</td>
</tr>
<tr>
<td>4</td>
<td>Molecular Grade Water</td>
<td>1 vial, 40µl</td>
</tr>
<tr>
<td>5</td>
<td>Internal Control</td>
<td>1 vial, 30µl</td>
</tr>
<tr>
<td>6</td>
<td>MP Positive Control</td>
<td>1 vial, 30µl</td>
</tr>
</tbody>
</table>

---

**Analysis sensitivity:** 1 TQ copy/reaction

Note: Analysis sensitivity depends on the sample volume, elution volume, nucleic acid extraction methods and other factors. If you use the DNA extraction buffer in the kit, 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°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.
- Reaction Mix should be stored in the dark.

6. Additional Required Materials and Devices

- **Biological cabinet**
- **Trypsin digestion Solution**
- **Neutralizing Solution**
- **Real time PCR system**
- **Vortex mixer**
- **Cryo-container**
- **Sterile pipettes tips for micro pipets**
- **Disposable gloves, powderless**
- **Refrigerator and Freezer**
- **Tube racks**
- **Desktop microcentrifuge for “eppendorf” type (RCP max. 16,000 x g)***

---

**Warnings and Precaution**

Carefully read this instruction before starting the procedure.

**For in vitro diagnostic use only:**

- This assay is not 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 is not 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 thawed, vortex and centrifuge briefly the tubes before use.
- Prepare quickly The reaction mixture and make sure the tubes are mixed.
- 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.
- Avoid cross contamination.

---

8. Sample Collection, Storage and transport

- Collect 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 DNA-Extraction

DNA extraction buffer is supplied in the kit.

**Attention:** please thaw the buffer thoroughly and mix the buffer well before use because it contains insoluble particles. You may use your own extraction systems or commercial kits.

9.1.1 Sputum sample

1) Trypsin Digestion Solution preparation
   Add 10g trypsin to 200ml sterile purified water and mix thoroughly. Adjust the PH value to 8.0 with 2 N NaOH solution. Add the PCR cycle with 0.4% CaCl₂, mix thoroughly and store at 4°C. Please incubate at 37°C for 10 minutes before use.

2) Estimate the volume of the sputum and add parts aequales of the trypsin digestive solution then vortex vigorously. Set at room temperature for 30 minutes. Transfer 0.5ml mixture to a new tube. Centrifuge the tube at 13000rpm for 5 minutes, carefully remove and discard supernatant from the tube without disturbing the pellet.

3) Add 1ml normal saline. Resuspend the pellet with vortex vigorously. Centrifuge at 13000rpm for 5 minutes. Carefully remove and discard supernatant from the tube without disturbing the pellet.

4) Repeat step 3)

5) Add 10µl DNA extraction buffer, closed the tube then resuspend the pellet with vortex vigorously. Spin down briefly in a table centrifuge.

6) Incubate the tube for 10 minutes at 100°C.

7) Centrifuge the tube at 13000rpm for 10 minutes. The supernatant contains the DNA extracted and can be used for PCR template.

9.1.2 Fluid samples (nasal and pharyngeal secretions and etc.)

1) Take 1ml sample in a tube, centrifuge the tube at 13000rpm for 2 minutes, and remove the supernatant and keep the pellet.

2) Add 10µl DNA extraction buffer to the pellet, close the tube then vortex for 10 seconds. Spin down briefly in a table centrifuge.

3) Incubate the tube for 10 minutes at 100°C.

4) Centrifuge the tube at 13000rpm for 10 minutes. The supernatant contains the DNA extracted and can be used for the template of the PCR.

---

10.4.3 Tissue sample

1) Wash the sample (lung biopsy) in 0.5ml normal saline and vortex vigorously. Centrifuge at 13000rpm for 2 minutes. Carefully remove and discard supernatant from the tube without disturbing the pellet.

2) Add 10µl DNA extraction buffer to the tube, close the tube then vortex for 10 seconds. Spin down briefly in a table centrifuge.

3) Incubate the tube for 10 minutes at 100°C.

4) Centrifuge the tube at 13000rpm for 10 minutes. The supernatant contains the DNA extracted and can be used for PCR template.

9.2 Internal Control

It is necessary to add internal control (IC) in the reaction mix. Internal Control (IC) allows the user to determine and control the possibility of PCR inhibition.

For use with ABI Prism™7000/7300/7500/7800/Step One Plus; iCycler iQ®+iQ5™; Smart Cycler II/Bio-Rad CFX 96; Rotorgene® 6000; Mx3000P/3050P; MJ-Option2/Chromo4; LightCycler®480 Instrument

Add the internal control (IC) 1µl/10µl and the result will be shown in the HEX/VIC/JOE.

---

9.3 PCR Protocol

**The Master Mix volume for each reaction should be pipetted as follows:**

1) Volumes of Reaction Mix and Enzyme Mix per reaction multiply with the number of samples, which includes the number of controls, standards, and sample prepared. Molecular Grade Water is used as the negative control. For reasons of unspecified pipetting, always add an extra virtual sample. Mix completely then spin down briefly in a centrifuge.

2) Pipet 9µl (2.5µl for Smart Cycler II) Master Mix with micropipets of sterile filter tips to each Real time PCR reaction plate/tubes. Separately add 4µl (2.5µl for Smart Cycler II) DNA sample, positive and negative controls to different reaction plates/tubes. Immediately close the plates/tubes to avoid contamination.

3) Spin down briefly in order to collect the Master Mix in the right reaction tube.

4) Perform the following protocol in the instrument:

**Result Analysis**

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.

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

**Ct value**

<table>
<thead>
<tr>
<th>FAM</th>
<th>HEX/VIC/JOE</th>
<th>Target Nucleic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>UNDET/25–35</td>
<td>Below the detection limit or negative</td>
</tr>
<tr>
<td>≤38</td>
<td>Positive</td>
<td></td>
</tr>
</tbody>
</table>

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