Vibrio Cholera Real Time PCR Kit User Manual

LT022300DD50

For use with ABI Prism® 7000/7300/7500/7900/Step One Plus; iCycler IQ® 48/485®; Smart Cycler II/Bio-Rad CFX 96/Rotor Gene® 6000; Mx3000P/3005P; MJ-Option® Chromo4, LightCycler®480 Instrument

1. Intended Use

Vibrio Cholera real time PCR kit is used for the detection of Vibrio Cholera in stool or water samples by using real time PCR systems.

2. Principle of Real-time PCR

Titrated sample of the real-time detection is based on the fluorogenic 5’nucleic assay. During the PCR reaction, the DNA polymerase cleaves the fluorophore 5’ triphosphate from the probe and releases the reporter 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 (Cq) is proportional to the amount of the specific PCR product. Monitoring the fluorescence intensities during real-time PCR allows the detection of the accumulating product without having to re-open the reaction tube after the amplification.

3. Product Description

Cholera is an acute intestinal infection caused by ingestion of food or water contaminated with the bacterium Vibrio cholera. It has a short incubation period and produces an enterotoxin that causes a profuse, painful, watery diarrhoea that can quickly lead to severe dehydration and death if treatment is not promptly given. Vomiting also occurs in most patients.

3.1 Cholera Symptoms

Most persons infected with V. cholerae do not become ill, although the bacterium is present in their faeces for 7-14 days. When illness does occur, about 80-90% of episodes are of mild or moderate severity and are difficult to distinguish clinically from other types of acute diarrhoea. Less than 20% of persons develop typical cholera signs with severe or moderate dehydration. Cholera remains a global threat and is one of the key indicators of social development. While the disease no longer poses a threat to countries with minimum standards of hygiene, it remains a challenge to countries where access to safe drinking water and adequate sanitation cannot be guaranteed.

4. Kit Contents

4.1 Type of reagent

<table>
<thead>
<tr>
<th>Type of reagent</th>
<th>Presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 DNA extraction buffer</td>
<td>1 vial, 30μl</td>
</tr>
<tr>
<td>2 Vibrio Cholera Reaction Mix</td>
<td>1 vial, 95μl</td>
</tr>
<tr>
<td>3 PCR Enzyme Mix</td>
<td>1 vial, 12μl</td>
</tr>
<tr>
<td>4 Molecular Grade Water</td>
<td>1 vial, 400μl</td>
</tr>
<tr>
<td>5 Internal Control (IC)</td>
<td>1 vial, 30μl</td>
</tr>
<tr>
<td>6 Vibrio Cholera Positive control (1×10^7 Copies/ml)</td>
<td>1 vial, 30μl</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.

6. Additional Required Materials and Devices

<table>
<thead>
<tr>
<th>Material/Device</th>
<th>Required</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biological safety cabinet</td>
<td>Yes</td>
</tr>
<tr>
<td>Vortex mixer</td>
<td>Yes</td>
</tr>
<tr>
<td>Cryo-container</td>
<td>Yes</td>
</tr>
<tr>
<td>Sterile filter tips for micro pipets</td>
<td>Yes</td>
</tr>
<tr>
<td>Disposable gloves, powder free</td>
<td>Yes</td>
</tr>
<tr>
<td>Refrigerator and freezer</td>
<td>Yes</td>
</tr>
<tr>
<td>Desktop centrifuge for “suspension” type tubes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

7. Warnings and Precautions

- Carefully read this instruction before starting the procedure.
- For in vitro diagnostic use only.
- This assay is in need a certified and qualified lab personnel.
- Clinical samples should be regarded as potentially infectious materials. They 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.
- Quickly prepare 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.

8. Sample Collection, Storage and Transportation

- 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. Please thaw the buffer thoroughly and spin down briefly in the centrifuge before use. It’s better to use commercial kits for nucleic acid extraction.

9.1.1 Stool Samples

1) Take about 50mg samples to a 1.5ml tube; add 1.0ml normal saline then vortex for 10 seconds.. Centrifuge the tube at 13000rpm for 2 minutes, carefully remove and discard supernatant from the pellet without disturbing the pellet.
2) Add 10μl DNA extraction buffer, close the tube then resuspend the pellet with vortex vigorously. Spin down briefly in a table centrifuge.
3) Incubate the tube for 10 minutes at 100°C.
4) Centrifuge the tube at 13000rpm for 5 minutes. The supernatant contains the DNA extracted and can be used for PCR template.

9.1.2 Water Samples

1) Take 3ml water to a tube. Centrifuge the tube at 13000rpm for 2 minutes, carefully remove and discard supernatant from the tube without disturbing the pellet.
2) Add 10μl DNA extraction buffer, 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 5 minutes. The supernatant contains the DNA extracted and can be used for PCR template.

10. Threshold setting:

The threshold for detection of Vibrio Cholera is defined as the cycle number at which the target DNA is detected.

11. Calibration of quantitative detection

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.

12. Quality control:

- Negative control (1×10^6 copies/ml) contains high concentration of the target DNA.
- Positive control:
  - 1×10^7 copies/ml
  - 1×10^6 copies/ml
  - 1×10^5 copies/ml
  - 1×10^4 copies/ml

13. Data Analysis and Interpretation

The following results are possible:

- Negative: Below the detection limit
- Positive: The result is below the detection limit
- Positive: The result is above the detection limit

- Cq (quantitative detection) = 10

- Coefficient of Correlation of Cq values: R = 0.98