Refrigeration and other factors. If you use the RNA extraction fluorescence of the internal control (IC).

contains a system to identify possible PCR inhibition by measuring the amplification of the Reaction) in the real time. Each size. Each death through dehydration. Although rotavirus was discovered in 1973 and a species of this virus, referred to as A, B, C, D, E, F and G Rotavirus A, the most common, causes more than 90% of infections in humans.

Rotavirus is transmitted by the fecal-oral route. It infects cells that line the small intestine and produces an enterotoxin, which induces gastroenteritis, leading to severe diarrhea and sometimes death through dehydration. Although rotavirus was discovered in 1973 and accounts for up to 50% of hospitalisations for severe diarrhoea in infants and children, its importance is still not widely known within the public health community, particularly in developing countries. In addition to its impact on human health, rotavirus also infects animals, and is a pathogen of livestock.

The genome of rotavirus consists of 11 unique double-stranded RNA molecules which are 18,555 nucleotide base-pairs in total. Each helix, or segment, is a gene, numbered 1 to 11 by decreasing size. Each gene codes for one protein, except genes 9 and 11, which each code for two. The RNA is surrounded by a three-layered icosahedral protein capsid. Viral particles are up to 76.5 nm in size.

1. Intended Use

Rotavirus (Group A) real time RT-PCR kit is used for the detection of Group A Rotavirus in stool or other samples 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 monitoring the fluorescence intensities during Real Time allows the detection of the accumulating product without open the reaction tube after the amplification.

The permision of the real-time 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 open the reaction tube after the amplification.

3. Product Description

Rotavirus is a genus of double-stranded RNA virus in the family Reoviridae. It is the leading cause of severe diarrhea among infants and young children. By the age of five, nearly every child in the world has been infected with rotavirus at least once. However, with each infection, immunity develops and subsequent infections are less severe. There are seven species of this virus, referred to as A, B, C, D, E, F and G. Rotavirus A, the most common, causes more than 90% of infections in humans.

Specimens can be extracted immediately or after storage at -80°C.

4. Kit Contents

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.
- Use Sterile pipette tips to filter tech to each of the final transferrin expression systems or the commercial kit based on the yield.

7. 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 caps and gloves in each area.
- Do not pipet 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 after freeze 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

Different brand RNA extraction kits are available. You may use your own extraction system 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>Nucleic Acid Isolation Kit</th>
<th>Cat. Number</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNA Isolation Kit</td>
<td>ME-0010/ME-0012</td>
<td>ZJ Biotech</td>
</tr>
<tr>
<td>QuantiFast Viral RNA Mini extraction Kit (50)</td>
<td>32004</td>
<td>QIAGEN</td>
</tr>
</tbody>
</table>

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.

Add the internal control (IC) inICx and the result will be got in the HEX/VIC/OE3 channel.

9.3 RT-PCR Protocol

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

- 10 µl Target Nucleic Acid
- HEX/VIC/OE3 fluorochrome of the internal control (IC)

5’ PCR system without HEX/VIC/OE3 channel may be treated with 1µl Molecular Grade Water instead of 3µl IC.

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

2) Pipet 20µl Master Mix with micropipettor or filter tip tech to each of the final Real time PCR reaction plates/tubes. Separately add 5µl RNA sample, positive and negative controls to different reaction plates/tubes. Immediately close the plate/tubes to avoid contamination.

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

4) Perform the following protocol in the instrument:

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Duration</th>
<th>Cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>95°C for 10min</td>
<td>1cycle</td>
<td>HEX/VIC/OE3 fluorochrome</td>
</tr>
<tr>
<td>95°C for 15sec, 60°C for 1min (Fluorescence measured at 60°C)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5) 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 results are possible:

<table>
<thead>
<tr>
<th>Control</th>
<th>Channel</th>
<th>C value</th>
<th>Result Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAM</td>
<td>HEX/VIC/OE3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molecular Grade Water</td>
<td>UNDET</td>
<td>25–35</td>
<td></td>
</tr>
<tr>
<td>Positive (contraluent assay)</td>
<td>UNDET</td>
<td>5/3</td>
<td></td>
</tr>
</tbody>
</table>

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

For In Vitro Diagnostic Use Only

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