1. Sensitivity is the same as it declares. However, when the sample volume is dozens or even hundreds of times.

2. Principle of Real-Time PCR

The principle of the real-time PCR reaction 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 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 is proportional to the amount of the specific PCR product. Monitoring the fluorescence intensities in real-time allows the detection of the specific PCR product without having to re-open the reaction tube after the amplification.

3. Product Description

Rabies Virus is the pathogen for Rabies (also known as “hydrophobia”). Most animals can be infected by the virus and can transmit the disease to humans. Infected bats, monkeys, raccoons, foxes, skunks, cattle, wolves, dogs or cats may provide the greatest risk. But Rodents (mice, squirrels etc.) are seldom infected. The virus is usually present in the nerves and saliva of a symptomatic rabid animal. The virus has a bullet-like shape with a length of about 180 nm and a cross-sectional diameter of about 75 nm. One end is round or conical and the other end is planar or concave. The genome is composed of the unsegmented RNA of about 12,000 nt. Rabies Virus real time RT-PCR kit contains a specific ready-to-use system for the detection of Rabies Virus using RT-PCR (Reverse Transcription Polymerase Chain Reaction) in the real-time PCR system. The master contains a SuperMix for the specific amplification of Rabies Virus RNA. The reaction is done in one step in real-time RT-PCR. The first step is a reverse transcription (RT), during which Rabies Virus RNA is transcribed into cDNA. Afterwards, a thermostable DNA polymerase is used to amplify the specific gene (polymersase chain reaction). Fluorescence is emitted and measured by the real time systems’ optical unit during the PCR. The amplification of amplified Viruses DNA fragment is performed in fluorimeter channel 530nm with the fluorescent quencher BHQ1. In addition, the kit contains a system to identify possible PCR inhibition by measuring the 560nm fluorescence of the internal control (IC). An external positive control defined as 1×10³ copies/ml is supplied which allow the determination of the gene load. For further information, please refer to section 9.3 Quantitation.

4. Kit Contents

Ref. Type of reagent Presentation 25x3ml

1 RABV Super Mix 1 vial, 350µl
2 RT-PCR Enzyme Mix 1 vial, 28µl
3 Molecular Grade Water 1 vial, 400µl
4 Internal Control (IC) 1 vial, 30µl
5 RABV Positive Control(1×10⁷ copies/ml) 1 vial, 30µl

Analysis sensitivity: 5×10³ copies/ml LLOQ:1×10⁰ ~ 1×10⁸ copies/ml

Note: Analysis sensitivity depends on the sample volume, elution volume, nucleic acid extraction methods and other factors. If you use the RNA extraction kits recommended, 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 linear until the expiration date indicated on the kit label.
• Repeat 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. Additionally Required Materials and Devices

- Biological cabinet
- Real time PCR system
- Desktop microcentrifuge for “eppendorf” type tubes (RCF max. 16,000 g)
- Vortex mixer
- RNA extraction kit
- Real time PCR reaction tubes/pipettes
- Cryo-container
- Pipets (0.5 µl – 1000 µl)
- Sterile filter tips for micro pipets
- Sterile micropipets
- Disposable gloves, powderless
- Biosafety waste container
- Refrigerator and freezer
- Tube racks

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 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 coats and gloves in each area.
- Do not pipeline by mouth. Do not eat, drink, smoke in laboratory.
- Avoid aerosol.

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

Different brand RNA extraction kits are available. 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:

Nucleic Acid Isolation Kit Cat. Number Manufacturer

10. Threshold setting: Choose Arithmetic as back ground and none as Noise Band method, then adjust the Noise Band just under the minimum of the positive control.

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: Negative control and QC sample must be performed correctly, otherwise the sample results is invalid.

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

10. Threshold setting: Choose Arithmetic as back ground and none as Noise Band method, then adjust the Noise Band just under the maximum of the positive control.

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: Negative control and QC sample must be performed correctly, otherwise the sample results is invalid.

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