Pseudorabies Virus Real Time PCR Kit

Cat. No.: AD-0105-02

For use with ABI Prism®7000/7300/7500/7900; Smart CyclerII; iCycler iQ™4/iQ™5; Rotor Gene™2000/3000; Mx3000P/3005P; MJ-Option2/Chromo4 Real Time PCR Systems.

For In Vitro Diagnostic Use Only

User Manual
1. Intended Use
Pseudorabies real time PCR kit is used for the detection of Pseudorabies in tissue or nasopharyngeal swab sample by using real time PCR systems.

2. Introduction
Pseudorabies is an acute, frequently fatal disease affecting most species of domestic and wild animals; however, man and the higher apes are resistant to it. The disease is caused by a herpes virus and is characterized by a variety of clinical signs; the most prominent involve the nervous and respiratory systems. Severe itching and self-mutilation are seen in most species, but rarely in swine. Pseudorabies is spread mainly by direct contact between swine. The nose and mouth are the main entry points for the virus. Nasal discharges and saliva contain the virus, so drinking water, bedding, and other objects such as clothing and instruments may become contaminated.

3. Principle of Real-Time PCR
The principle of the real-time detection 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 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 (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.

4. Product Description
Pseudorabies virus real time PCR kit contains a specific ready-to-use system for the detection of Pseudorabies virus by polymerase chain reaction in the real-time PCR system. The master contains reagents and enzyme for the specific amplification of Pseudorabies virus DNA. Fluorescence is emitted and measured by the real time systems’ optical unit. The detection of amplified Pseudorabies virus DNA fragment is performed in fluorimeter channel FAM with the fluorescent quencher BHQ1. DNA extraction buffer is available in the kit and tissue or nasopharyngeal swab samples are used for the extraction of the DNA. In addition, the kit contains a system to identify possible PCR inhibition by measuring the VIC/JOE fluorescence of the internal control (IC). An external positive control (1x10^7 copies/ml) contained, allows the determination of the gene load. For further information, please refer to section 10.3 Quantitation.

5. 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, 1.8ml</td>
</tr>
<tr>
<td>2</td>
<td>Pseudorabies Reaction Mix</td>
<td>1 vial, 950µl</td>
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<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, 400µl</td>
</tr>
<tr>
<td>5</td>
<td>Internal Control (IC)</td>
<td>1 vial, 30µl</td>
</tr>
<tr>
<td>6</td>
<td>Pseudorabies Positive Control(1x10^7 copies/ml)</td>
<td>1 vial, 30µl</td>
</tr>
</tbody>
</table>

6. 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.

7. Additionally Required Materials and Devices
- Biological cabinet
- *Real time* PCR system
- Desktop microcentrifuge for “eppendorf” type tubes (RCF max. 16,000 x g)
- Vortex mixer
- *Real time* PCR reaction tubes/plates
- Cryo-container
- Pipets (0.5 µl – 1000 µl)
- Sterile filter tips for micro pipets
- Sterile microtubes
- Disposable gloves, powderless
- Biohazard waste container
- Refrigerator and Freezer
- Tube racks

8. 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 coats and gloves in each area.
- Do not pipette by mouth. Do not eat, drink, smoke in laboratory.
- Avoid aerosols

9. 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

10. Procedure
10.1 DNA-Extraction
DNA extraction buffer is supplied in the kit, please thaw the buffer thoroughly and spin down briefly in the centrifuge before use.
10.1.1 Tissue sample
1) Take 50mg sample to a tube, add 50µl DNA extraction buffer, close the tube then vortex for 10 seconds. Spin down briefly in a table centrifuge.
2) Incubate the tube for 10 minutes at 100°C.
3) Centrifuge the tube at 13000rpm for 10 minutes. The supernatant contains the DNA extracted and can be used for PCR template.

10.1.2 Nasopharyngeal swab sample
1) Take 1ml sample in a tube, centrifuge the tube at 13000rpm for 2min, and remove the supernatant and keep the pellet.
2) Add 50µl 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 PCR template.

Attention:
A. During the incubation, make sure the tube is not open, as the vapor will volatilize into the air and may cause contamination in case the sample is positive.
B. The extraction sample should be used in 3 hours or store at -20°C for one month.
C. Different brand of DNA extraction kits are available. The customer can use your own extraction systems or the commercial kit based on the yield. For DNA extraction, please comply with the manufacturer’s instructions.

10.2 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) 1µl/rxn and the result will be shown in the VIC/JOE channel.

10.3 Quantitation
The kit can be used for quantitative or qualitative real-time PCR.
For performance of quantitative real-time PCR, Standard dilutions must prepare first as follows.
Molecular Grade Water is used for dilution.

Take positive control (1×10^7 copies/ml) as the starting high standard in the first tube. Respectively pipette 36ul Molecular Grade Water into next three tubes. Do three dilutions as the following figures:

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.
Dilution is not need for performance of qualitative real-time PCR.

**Attention:**

A. Mix thoroughly before next transfer.

B. The positive control (1×10\(^7\) copies/ml) contains high concentration of the target DNA. Therefore, be careful during the dilution in order to avoid contamination.

### 10.4 PCR Protocol

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

1. The 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 unprecise pipetting, always add an extra virtual sample. (\(n\): the number of reaction)

<table>
<thead>
<tr>
<th>Reaction Volume</th>
<th>Master Mix Volume</th>
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<tbody>
<tr>
<td>35µl Reaction Mix</td>
<td>35µl × (n+1)</td>
</tr>
<tr>
<td>0.4µl Enzyme Mix</td>
<td>0.4µl × (n+1)</td>
</tr>
<tr>
<td>1µl Internal control (IC)</td>
<td>1µl × (n+1)</td>
</tr>
</tbody>
</table>

Mix completely then spin down briefly in a centrifuge.
2) Pipet **36µl** Master Mix with micropipets of sterile filter tips to each Real time PCR reaction plate/tubes. Separately add **4µl** DNA sample, positive and negative controls to different reaction plate/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:

   
   \[
   \begin{align*}
   &37^\circ C \text{ for 2 min, 1 cycle;} \\
   &94^\circ C \text{ for 2 min, 1 cycle;} \\
   &93^\circ C \text{ for 15 sec, } 60^\circ C \text{ for 60 sec, 40 cycles.}
   \end{align*}
   \]

   **Fluorescence is measured at 60°C**

11. **Data Analysis and Interpretation**

The following results are possible:

1) A signal is detected in channel FAM. **The result is positive: The sample contains Pseudorabies virus DNA.**

   In this case, the detection of a signal in channel VIC/JOE (Internal control) is dispensable, as high initial concentrations of Pseudorabies virus DNA can lead to a reduced or absent fluorescence signal of the internal control (competition).

2) In channel FAM no signal is detected, at the same time, a VIC/JOE signal from the Internal Control appears. **The sample does not contain any Pseudorabies virus DNA. It can be considered negative.**

   In the case of a negative Pseudorabies virus PCR the detected signal of the internal control rules out the possibility of PCR inhibition.

3) Neither in channel FAM nor in channel VIC/JOE is a signal detected. **A diagnostic statement can not be made.** Inhibition of the PCR reaction.

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