Porcine Japanese Encephalitis Virus Antibodies ELISA Kit
Catalog No: E-AD-E002
96T

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Phone: 240-252-7368(USA)       Fax: 240-252-7376(USA)
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.
Test principle
This kit is comprised by ELISA Microtiter plate pre-coated with recombinant Porcine Japanese Encephalitis Virus (JEV) E2 protein, HRP conjugate and other auxiliary reagents, and apply the principle of enzyme-linked immunoassay (ELISA) to detect porcine JEV antibody of porcine serum, plasma samples. During the experiment, add control serum and samples into the ELISA Microtiter plate. If JEV specific antibodies exist in the samples after incubation, it will be bound with the recombinant protein on the micro-plate. Then wash the plate to remove unbound antibodies and other components, add the HRP conjugate to specifically bind with the compound of antibody and antigen on the Micro-plate. The unbound HRP conjugate will be removed by washing. TMB is added into the well, it will react with the enzyme and the products become blue. The color shade is of positive correlation with specific antibody levels in the samples. At last, end the reaction by adding stop solution to produce a yellow product. Measure the absorbance value of each well by using a Micro-plate Reader with 450 nm wavelength, then we can know whether there are porcine JEV antibody in the samples.

Kit components
<table>
<thead>
<tr>
<th>Item</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA Microtiter plate</td>
<td>96 wells</td>
</tr>
<tr>
<td>Dilution plate</td>
<td>96 wells</td>
</tr>
<tr>
<td>HRP Conjugate</td>
<td>11 mL</td>
</tr>
<tr>
<td>Sample Diluent</td>
<td>50 mL</td>
</tr>
<tr>
<td>20xConcentrated Wash Buffer</td>
<td>40 mL</td>
</tr>
<tr>
<td>Substrate Reagent A</td>
<td>6 mL</td>
</tr>
<tr>
<td>Substrate Reagent B</td>
<td>6 mL</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>6 mL</td>
</tr>
<tr>
<td>Positive Control</td>
<td>1 mL</td>
</tr>
<tr>
<td>Negative Control</td>
<td>1 mL</td>
</tr>
<tr>
<td>Plate Sealer</td>
<td>3 pieces</td>
</tr>
<tr>
<td>Sealed Bag</td>
<td>1 piece</td>
</tr>
<tr>
<td>Manual</td>
<td>1 copy</td>
</tr>
</tbody>
</table>

Experimental instrument
Microplate Reader with 450 nm wavelength filter or dual-wavelength (450/630 nm)
High-precision transferpettor, EP tubes and disposable pipette tips
37°C incubator or water bath
Deionized or distilled water
Absorbent paper
Sample preparation
1. Use the conventional method to prepare serum/plasma, the serum/plasma must be clear, no hemolysis and no pollution. Samples can be conserved at 2~8℃ in 1 week, and it should be stored at -20℃ for a long term storage.
2. Dilute the sample serum/plasma with the sample diluent at 1:39 (5 μL sample serum/plasma and 195 μL of sample diluent, mix properly). The positive/negative control do not need to be diluted.
3. The 20× Concentrated wash buffer should be adjusted to room temperature before use, then dilute it with distilled or deionized water at 1:19.
4. Bring all reagents to room temperature (18~25℃) for 30 min before use.

Assay procedure
1. **Number:** Take out the micro-plate, set 1 well for blank control and 2 wells for negative/positive control respectively. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2~8℃. Double well parallel experiment is recommended for detection.
2. **Add sample:** Add 100 μL of sample diluent to the blank control well, add 100 μL of positive/negative control to positive/negative control well, and add 100 μL of diluted sample to the sample wells.
3. **Incubate:** Gently tap the plate to ensure thorough mixing, incubate at 37℃ for 30 min.
4. **Wash:** Aspirate each well and wash, repeat the process 5 times, immerse 30-60 sec each time. Wash by filling each well with wash buffer (approximately 300 μL). Complete removal of liquid at each step is essential. After the last wash, remove remained wash buffer by aspirating or decanting. Invert the plate and pat it against thick clean absorbent paper.
5. **HRP conjugate:** Add 100 μL of HRP conjugate into each well (except the blank control well), and incubate at 37℃ for 30 min.
6. **Wash:** Repeat step 4 for washing.
7. **Color Development:** Add 50 μL of substrate reagent A and 50 μL of substrate reagent B into each well, gently tap the plate to ensure thorough mixing, incubate for 10 min at 37℃. Protect from light.
8. **Stop reaction:** Add 50 μL of stop solution into each well, gently mix.
9. **OD Measurement:** Adjusted zero with the blank control, measure the absorbance value (A-value) of each well by using a Micro-plate reader with 450 nm wavelength (or use 630 nm as reference wavelength).
Reference value
Normally, the A-value of negative control ≤ 0.1 and positive control ≥ 0.6

Interpretation of the results
1. Positive result: $A_{450} \geq 0.38$
2. Suspicious result: $0.38 > A_{450} \geq 0.2$
3. Negative result: $A_{450} < 0.2$
4. The negative result of this test suggests that the concentration of antibody for the tested porcine is not enough, it is recommended that this porcine should get immunized with corresponding vaccine.

Limitations of this test method
This test is only used as the qualitative detection of JEV antibodies in porcine serum and plasma. A rough estimate (high, general, low) of the antibody concentration can be calculated according to the A values.

Notes
1. Wear gloves and work clothes during experiment, and the disinfection and isolation system should be strictly performed. All the waste should be handled as contaminant.
2. The stop solution is corrosive, it should be avoided to contact with skin and clothing. Wash immediately with plenty of water if contacted carelessly.
3. The ELISA plate obtained from cold storage conditions should be adjusted to room temperature before opening the bag. The unused plate should be kept in a sealed bag with desiccant.
4. Concentrated wash buffer at low temperature condition is easy to crystallize, it should be adjusted to room temperature in order to dissolve completely before use.
5. Each well must be filled with liquid when washing in order to prevent residual free enzyme.
6. The tested sample should keep fresh.
7. The results shall depend on the readings of the microplate reader.
8. Do not use components from different batches of kit.

Storage and Expiry date
Store at 2~8°C with shading light for 12 months.
Please store the opened plate at 2~8°C, protect from light and moisture. The valid period is 2 months.
Valid Period: expiration date is on the packing box.