Porcine Transmissible Gastroenteritis Virus Antibodies ELISA Kit
Catalog No: E-AD-E041
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 ELISA kit is only used as the qualitative detection of Porcine Transmissible Gastroenteritis Virus (TGEV) antibody in serum or plasma samples in vitro. The ELISA Microtiter plate provided in this kit has been pre-coated with the TGEV recombinant N protein. Control solution and samples are added into Microplate and incubate. If TGEV antibody exist in the samples, it will be bound with the antigen pre-coated on the microplate. Then wash to remove unbound antibodies and other components, add the HRP Conjugate to specifically bind with the compound of antibody and antigen on the Microplate. The unbound HRP conjugate will be removed by washing. Add TMB substrate in the wells, it will react with the enzyme and become blue, the shade of color is of positive correlation with TGEV antibody levels in the samples. Finally, end the reaction by adding stop solution to produce a yellow product. Measure the absorbance value of each well at 450 nm with a Microplate Reader, then the presence of TGEV antibody can be determined.

Kit components

<table>
<thead>
<tr>
<th>Item</th>
<th>Specifications</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>20×Concentrated 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>Dilute plate</td>
<td>1 piece</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 450nm wavelength filter or dual-wavelength (450/630nm)
High-precision transferpettor, EP tubes and disposable pipette tips
37°C incubator or water bath
Deionized or distilled water
Absorbent paper
**Reagent preparation**

1. Use the conventional method to prepare serum or plasma, the samples must be clear, no hemolysis and no pollution. Samples can be stored at 2~8°C in 1 weeks or -20°C for a long term storage.
2. Dilute the sample with the sample diluent at 1:39 (5 μL of sample and 195 μL of sample diluent and mix fully). The positive/negative control do not need to be diluted.
3. The 20×Concentrated Wash Buffer should be adjusted to room temperature before used, then dilute it with deionized or distilled water at 1:19.
4. Bring all reagents to room temperature (18~25°C) 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°C. 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. Add 100 μL of diluted sample to the sample wells.
3. **Incubate:** Gently tap the plate to mix thoroughly and incubate at 37°C for 30 min.
4. **Wash:** remove the liquid in each well. Immediately add 300 μL of wash buffer to each well and wash. Repeat wash procedure for 5 times, 30 s intervals/time. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
5. **HRP conjugate:** Add 100 μL of HRP conjugate into each well (except the blank control well) and incubate at 37°C 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 mix thoroughly. Incubate at 37°C for 10 min with shading light.
8. **Stop reaction:** Add 50 μL of Stop Solution into each well and mix thoroughly.
9. **OD Measurement:** Determine the absorbance (A-value) of each well at 450 nm with a micro-plate reader (the 450/630 nm double wavelength is recommended). This step should be finished in 10 min after stop reaction.
Reference value
Normally, the A-value of negative control ≤ 0.1 and A-value of 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 TGEV antibody in porcine of serum and plasma. A rough estimate (high, general, low) of the concentration 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 use. 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 be kept 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.