Foot and Mouth Disease Virus Asia I Antibodies ELISA Kit  
Catalog No: E-AD-E020  
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 FMDV-Asia-Ⅰ antibodies, Enzyme Conjugate and other auxiliary reagents, and apply the principle of Inhibition-ELISA to detect FMDV-Asia-Ⅰ antibody in serum of porcine, cattle, goat, sheep. During the experiment, add Antigen Solution and samples into the ELISA Microtiter plate. Antibodies in sample will compete with antibody pre-coated on the Micro-plate for the antigen and block the combination between the antigen and the Microplate. Then wash the plate to remove unbound antibodies and other components, add the Antibody Working Solution to incubate the reacting system. After washing, add the Enzyme Conjugate to specifically bind with the compound of antibody and antigen on the Micro-plate. The unbound Enzyme Conjugate will be removed by washing. Substrate is added into the well, it will react with the enzyme and the product become blue. The color shade is of negative 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 FMDV-Asia-Ⅰ antibodies 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>5.5 mL</td>
</tr>
<tr>
<td>Antibody Work Solution</td>
<td>5.5 mL</td>
</tr>
<tr>
<td>Antigen Solution</td>
<td>5.5 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>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°C in 1 week, and it should be stored at -20°C for a long term storage.

2. The 20×Concentrated Wash Buffer should be adjusted to room temperature before use, then dilute it with distilled or deionized water at 1:19.

3. Dilute the sample serum with the diluted Wash Buffer at 1:32 (5 μL sample serum and 155 μL of Wash Buffer, mix properly). The positive/negative control do not need to be diluted.

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 2 wells for negative /positive control respectively. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 4°C. Double well parallel experiment is recommended for detection.

2. **Add sample:** Add 50 μL of positive /negative control to positive/negative control wells. Add 25 μL of diluted wash buffer and add 25 μL of diluted sample to the sample wells. Then add 50 μL of Antigen Solution to each well.

   **Note:** The dilution factor is 1:128 after adding antigen.

3. **Incubate:** Gently tap the plate to ensure thorough mixing, incubate at 37°C for 30 min.

4. **Wash:** Aspirate each well and wash, repeat the process 5 times, immerse 30-60 seconds each time. Wash by filling each well with Wash Buffer (approximately 300 μL) (a squirt bottle, multi-channel pipette, manifold dispenser or automated washer are needed). 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. **Antibody Working Solution:** Add 50 μL of Antibody Working Solution into each well (except the blank control well), and incubate at 37°C for 30 min.

6. **Wash:** Repeat Step 4 for washing.

7. **HRP conjugate:** Add 50 μL of HRP Conjugate into each well (except the blank control well), and incubate at 37°C for 30 min.

8. **Wash:** Repeat Step 4 for washing.

9. **Color Development:** Add 50 μL of substrate A and 50 μL of substrate B into each well, gently tap the plate to ensure thorough mixing, incubate for 15 min at 37°C. Protect from light.

10. **Stop reaction:** Add 50 μL of Stop Solution into each well, gently mix.

11. **OD Measurement:** Measure the absorbance value (A-value) of each well by using a Microplate Reader in 450 nm wavelength (use 630nm as reference wavelength).
Reference value
Normally, the A-value of negative control ≥1.0 and A-value of positive control ≤ 50% × A-value of negative control.

Interpretation of the results
1. PI (Blockage rate) = (1−value of sample A/average A value of negative controls) ×100%, PI ≥ 50% it’s positive, PI < 50% it’s negative.
2. The positive result of this detection suggests that the sample contains FMDV-Asia-Ⅰ antibody, and the titer is more than 1:128, which has reached the protective level.
3. The negative result of this detection suggests that there is no FMDV-Asia-Ⅰ antibody in the sample, or the titer is less than 1:128, which has not reached the protective level.
4. To know the final titer of antibody in sample, dilute the sample with gradient dilution. The highest dilution ratio which making the PI≥50% is the final titer of antibody in this sample.

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

Notes
1. Wear gloves and work clothes during experiment, and the disinfection and isolation system should be strictly executed. 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 contact it carelessly.
3. Adjust the reagents and Micro-plate to room temperature before use. The unused Micro-plate should be stored at 2~8℃ in the valve 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℃ with shading light for 12 months.
Please store the opened plate at 2~8℃, protect from light and moisture. The valid period is 2 months.
Expiration date: expiration date is on the packing box.