Technical Manual

Foot and Mouth Disease Virus Type O Antibodies ELISA Kit

- Catalogue Code: ADES0011
- Antibody ELISA Kit
- Research Use Only

1. Test principle

This kit is comprised by HRP conjugate, other auxiliary reagents and ELISA Microtiter plate pre-coated with recombinant Foot and Mouth Disease Virus Type O (FMD-O) protein. Apply the principle of enzyme-linked immunoassay (ELISA) to detect porcine FMD-O antibody in serum of swine, cattle, goat, and sheep. During the experiment, add control serum and samples into the ELISA Microtiter plate. If FMD-O antibodies exist in the samples, it will be bound with the recombinant protein on the microtiter plate after incubation. 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 microtiter plate. The unbound HRP conjugate will be removed by washing. Substrate Reagent is added into the well, it will react with the enzyme and the products become blue. The color shade is of negative correlation with 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 microtiter plate Reader with 450 nm wavelength, then we can know whether there are FMD-O antibody in the samples.

2. Kit components

Item	Specifications
ELISA Microtiter plate	96 wells
HRP Conjugate	6 mL
20×Concentrated Wash Buffer	40 mL
Antigen Solution	6 mL
Antibody Working Solution	6 mL
Substrate Reagent A	6 mL
Substrate Reagent B	6 mL
Stop Solution	6 mL
Positive Control	1 mL
Negative Control	1 mL
Plate Sealer	3 pieces
Sealed Bag	1 pieces
Manual	1pieces

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

3. 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

4. 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. FOR RESEARCH USE ONLY. ELISA Microtiter plate should be covered by plate sealer. A void the kit to strong light.
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. **Each reagent is optimized for use in the ADES0011. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other ADES0011 with different lot numbers.**
9. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.

5. Storage and expiry date

Store at 2-8° C. Avoid freeze.
Please store the opened plate at 2-8° C, the shelf life of the opened kit is up to 1 month.
Expiry date: expiration date is on the packing box.

6. Sample pretreatment

1. **Serum**: Use the conventional method to prepare serum, the serum 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. **Wash Buffer**: The **20×Concentrated Wash Buffer** should be adjusted to room temperature to make the sediment dissolved fully before use, then dilute it with deionized water at 1:19.
3. **Sample Dilution**：The serum of cattle and sheep to be tested was diluted 32 times with the **Wash Buffer** (for example, 5μl serum was added to 155μl **Wash Buffer**, mixed), and the serum of pigs to be tested was diluted 16 times (for example, 5μl serum was added to 75μl **Wash Buffer**, mixed), and the negative positive control product did not need to be

diluted.

7. Assay procedure

Restore all reagents and samples to room temperature before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2-8° C.

1. **Number:** number the sample and control in order (multiple well), and keep a record of control wells and sample wells. Take out the microtiter plate, set 2 well for positive control and 2 wells for negative control. **Samples need test in duplicate.**
2. **Add sample:** add 50 μL of **positive control** and 50 μL of **Antigen Solution** to positive control well**,** add 50 μL of **negative control** and 50 μL of **Antigen Solution** to negative control well, and add 25 μL of **diluted serum,** 25 μL of **Wash Buffer** and 50 μL of **Antigen Solution** to sample well, mix fully.
3. **Incubate:** cover the plate sealer and mix thoroughly, incubate at 37° C for 30 min in shading light.
4. **Wash:** remove the liquid in each well. Immediately add 350 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. **Antibody Working Solution:** add 50 μL of **Antibody Working Solution** into each well, cover the plate sealer and incubate at 37° C for 30 min in shading light.
6. **Wash:** Repeat step 4 for washing
7. **HRP conjugate:** add 50 μL of **HRP Conjugate** into each well, cover the plate sealer and incubate at 37° C for 30 min in shading light.
8. **Wash:** Repeat step 4 for washing.
9. **Color Development:** add 50 μL of **Substrate Reagent A** and 50 μL of **Substrate Reagent B** into each well and mix thoroughly, cover the plate sealer and incubate for 15 min at 37° C in shading light.
10. **Stop reaction:** add 50 μL of **Stop Solution** into each well, mix thoroughly.
11. **OD measurement:** Measure the OD-value of each well by using a microplate reader with 450 nm wavelength (use 630 nm as reference wavelength). This step should be finished in 10 min after stop reaction.

8. Reference value

Normally, OD value of negative control (NC) ≥ 0.8 and OD value of positive control (PC) ≤ 50% * OD (NC).

9. Interpretation of the results

1. PI (Percentage of inhibition) = 1- (OD $_{sample}$ / Average OD $_{negative}$) ×100%
2. Positive result: PI ≥ 50%; Negative result: PI < 50%
3. Unimmunized animal: positive result indicates that it may be infected.

10. Limitations

1. This kit can only be used to qualitatively detect the FMDV-O antibody in serum of cattle, goat, sheep and swine. A rough estimate (high, general, low) of the antibody concentration can be calculated according to the PI value.
2. The detection results of this kit are only for reference. For confirmation of the result, please combine the symptoms and other methods of detection, this detection cannot be used as the only criteria for result.

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