Technical Manual

Foot and Mouth Disease Virus Type A Antibodies ELISA Kit

- Catalogue Code: ADES0012
- Antibody ELISA Kit
- Research Use Only

1. Test principle

This ELISA kit is consisted of Micro-plate pre-coated with FMDV-A antibody, Antibody Working Solution, HRP Conjugate, Antigen Solution and other reagents. It applies Inhibition-ELISA as the method for the detection of FMDV-A antibody in Cattle, Goat, Sheep, Porcine samples. Diluted samples and antigen are added into the Micro-plate and incubate. 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 Micro-plate. Then wash and add the Antibody Working Solution to incubate. Wash and add HRP Conjugate, incubate. Then wash away the unbound HRP Conjugate. Add TMB substrate to the wells, it will react with the enzyme and become blue, the shade of color is of negative correlation with the content of specific antibody 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 the presence of FMDV-A antibody can be determined.

2. Kit components

Item	Specifications
ELISA Microtiter plate	96 wells
Dilution plate	96 wells
Antigen Solution	5.5 mL
Antibody Working Solution	5.5 mL
HRP Conjugate	5.5 mL
20×Concentrated Wash Buffer	40 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 piece
Manual	1 copy

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 ADES0012. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other ADES0012 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. Use the conventional method to prepare serum/plasma, the serum/plasma must be clear, no hemolysis and no pollution. Samples can be stored at 2-8° C for one week, and it should be stored at -20° C for a long term storage.
2. Adjust the 20×Concentrated Wash Buffer to room temperature before use, then dilute it with distilled or deionized water at 1: 19.
3. Dilute the tested serum with the diluted Wash Buffer at 1:31 (5 μL of sample serum and 155 μL of Wash Buffer, mix fully). The positive/negative control does not need to be diluted.
4. Bring all reagents to room temperature (18-25° C) for 30 min before use.

7. 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 2-8° C. Double well parallel experiment is recommended for detection.
2. **Add Sample:** Add 50 μL of positive/ negative control to positive/negative control well respectively. 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:** Mix thoroughly for 10 sec, 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. **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 Reagent A and 50 μL of Substrate Reagent B into each well, mix thoroughly. Incubate for 15 min at 37° C in the dark.
10. **Stop reaction:** Add 50 μL of Stop Solution in each well, mix thoroughly.
11. **OD Measurement:** Measure the absorbance value (A-value) of each reaction well measured at 450 nm wavelength (use 630 nm as reference wavelength).

8. Reference value

Normally, the OD of negative control ≥ 1.0 and A-value of positive control ≤ 50% ×A-value of negative control.

9. Analysis of results

1. PI (Percentage of inhibition) = (1-Sample OD/Average of negative control ODs) × 100%.
2. Positive result : PI ≥ 50%
3. Negative result : PI ＜ 50%
4. The positive result of this detection suggests that the sample contains FMDV-A antibody, and the titer is more than 1:128, which has reached the protective level.
5. The negative result of this detection suggests that there is no FMDV-A antibody in the sample, or the titer is less than 1:128, which has not reached the protective level.

6. 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.

10. Limitations

This kit can only be used to qualitatively detect the FMDV-A antibody in serum of Cattle, Goat, Sheep, Porcine A rough estimate (high, general, low) of the antibody concentration can be calculated according to the PI value.

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