Technical Manual

Cattle and Goat Foot and Mouth Disease Virus Type O Antibodies ELISA Kit

- Catalogue Code: ADES0022
- Antibody ELISA Kit
- Research Use Only

1. Test principle

This kit is comprised by HRP conjugate, other auxiliary reagents, ELISA Microtiter plate pre-coated with FMDV-O antigen. Apply the principle of enzyme-linked immunoassay (ELISA) to detect FMDV-O antibody in serum and plasma of cattle. During the experiment, add control serum and samples into the ELISA Microtiter plate. If FMDV-O antibodies exist in the samples, it will compete with the antibody in the antibody working solution to bind with the antigen pre-coated on the Microplate. 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 become blue. The color shade is negative correlation with FMDV-O 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 Microplate Reader with 450 nm wavelength, then we can know whether there are FMDV-O antibodies in the samples.

2. Kit components

Item	Specification
ELISA Microtiter plate	96 wells
HRP Conjugate	11 mL
20×Concentrated Wash Buffer	40 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 piece
Manual	1 copy

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

3. 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

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. 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. **Each reagent is optimized for use in the ADES0022. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other ADES0022 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 preparation

1. **Serum/plasma**：Use the conventional method to prepare animal 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. **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.

7. Assay procedure

Restore all reagents and samples to room temperature (25° C) 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. Set 2 wells for negative/positive control respectively. **Samples need test in duplicate.**
2. **Add sample:** add 50 μL of **positive /negative control** to positive/negative control wells. Add 5 μL of **serum/plasma** and 45 μL of **Wash Buffer** to each sample well.
3. **Incubate:** add 50 μL of **Antibody Working Solution** to each well. 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 300 μL of **Wash Buffer** to each well and wash. Repeat wash procedure for 5 times, 30s 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, cover the plate sealer and incubate at 37° C for 30 min in shading light.
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. Cover the plate sealer and mix thoroughly, incubate at 37° C for 15 min in shading light.
8. **Stop reaction:** add 50 μL of **Stop Solution** into each well, mix thoroughly.
9. **OD Measurement:** measure the absorbance value (A-value) of each well by using a Microplate Reader with 450 nm wavelength (use 630 nm as reference wavelength).

8. Reference value

Normally, the A-value of negative control ≥0.8 and A-value of positive control ≤ 50% ×A-value of negative control.

9. Interpretation of the results

10. Analysis of results

1. PI (Percentage of inhibition) = (1- OD_{Sample} / Average $OD_{negative\ control}$) × 100%.
2. Positive result : PI ≥ 50% ; Negative result : PI ＜ 50%
3. Unimmunized animal: positive result indicates that it may be infected with FMD-O.
   Immunized animal：The antibody levels at the time of the sample were monitored and

recorded, and the distribution of antibody levels and the trend of immune status of the flock were analyzed based on the results.

11. Limitations of this test method

1. This kit is only used as the qualitative detection of FMDV-O antibodies in serum and plasma of cattle, sheep and goat. A rough estimate (high, general, low) of the antibody concentration can be calculated according to the PI values.
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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