Technical Manual

Foot and Mouth Disease Virus NSP Antibodies ELISA Kit

- Catalogue Code: ADES0026
- 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 the foot and mouth disease virus NSP (FMD-NSP) antigen. Apply the principle of enzyme-linked immunoassay (ELISA) to detect FMD-NSP antibody in serum of swine, cattle, goat, and sheep. During the experiment, add control serum and samples into the ELISA Microtiter plate. If FMD-NSP 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 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 FMD-NSP 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 judge whether FMD-NSP antibody exist in the sample.

2. Kit components

Item	Specification
ELISA Microtiter plate	96 wells
Sample Diluent	30 mL
10×Concentrated Wash Buffer	100 mL
HRP Conjugate	11 mL
Substrate Reagent	11 mL
Stop Solution	15 mL
Negative Control	2 mL
Positive 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. Other materials required but not supplied

- Microplate Reader with 450 nm wavelength filter or dual-wavelength (450/630 nm)
- High-precision transferpettor: Single channel (10-100 μL, 100-1000 μL)
- Measuring Cylinder: 500mL, EP tubes and disposable pipette tips
- 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 ADES0026. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other ADES0026 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:** Use the conventional method to prepare animal serum, the serum must be clear, no hemolysis and no pollution .Samples can be stored at 2-8° C for 1 week or - 20° C for a long term storage.
2. **Wash Buffer:** The **10×Concentrated Wash Buffer** should be adjusted to room temperature before use, then dilute it with deionized water at 1:9.

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. Take out the microtiter plate, set 1 well for positive control and 2 wells for negative control. **Samples need test in duplicate.**
2. **Add sample:** add 100 μL of **positive/negative control** to positive/negative control well. Add 80 μL of **Sample Diluent** and 20 μL of **Serum** to the sample wells, mix fully.
3. **Incubate:** cover the plate with sealer and mix thoroughly, incubate overnight at 22° C. (need 16-18 h).
4. **Wash:** remove the liquid in each well. Immediately add 250 μL of **Wash Buffer** to each well and wash. Repeat wash procedure for 6 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, and incubate at 22° C for 60 min in shading light.
6. **Wash:** Repeat step 4 for washing.
7. **Color Development:** add 100 μL of **Substrate Reagent** into each well and mix thoroughly. Incubate for 15 min at 37° C in shading light.
8. **Stop reaction:** add 50 μL of **Stop Solution** into each well, mix thoroughly.
9. **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 (N) > 0.6 and PI of positive control >50%.

9. Interpretation of the results

1. PI (Percentage of inhibition) = 1- (OD_{sample} / Average $OD_{negative}$) ×100%.
2. Positive result: PI > 50%.
3. Negative result: PI ≤ 50%.

10. Limitations of this test method

1. This kit is only used as the qualitative detection of FMD-NSP antibodies in animal (swine, cattle, goat, and sheep) serum.

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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