Bovine Ovine Caprine Foot and Mouth Disease Virus Type A (FMD-A Ab) Antibody ELISA Kit

Technical Manual

(ELISA)

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Product Information

Intended Use

Foot and Mouth Disease (FMD) is an acute, febrile, highly contagious disease of cloven-hoofed animals caused by the Foot and Mouth Disease Virus (FMDV). It is characterized by the formation of vesicles and erosions on the oral mucosa, feet, and mammary skin.

This assay is designed to detect antibodies against Foot and Mouth Disease Virus Type A (FMD-A) in the serum of bovine, sheep and goats. It can be used for the evaluation of the immunological efficacy of the FMD-A vaccine.

Principle and Application

This kit comprises a coated Microtiter Plate with FMD-A antigen, antibody solution, HRP conjugate, and other accompanying reagents. It employs the principle of enzyme-linked immunosorbent assay to detect antibodies against FMD-A in the serum/ plasma of bovine, sheep and goats. During the experiment, test sample and antibody solution are added to the plate. After incubation and subsequent washing to remove unbound components, HRP conjugate are added, which specifically binds to the antigen-antibody complexes on the plate. Further washing steps remove unbound HRP conjugate. Substrate reagents are then added to the wells. If FMD-A antibodies are present in the sample, they will inhibit the binding of the antibody solution to the antigens coated on the plates, preventing color development in subsequent reactions. Conversely, color will develop if no FMD-A antibodies are present. The intensity of the color is inversely proportional to the concentration of specific antibodies in the sample. The reaction is terminated by adding the stop solution, turning the product yellow. Absorbance is measured at 450 nm using a microplate reader to determine the presence or absence of FMD-A antibodies in the sample.

Composition of the Kit

Reagent	Specification		
Microtiter Plate	96wells	96wells×2	96wells×5
HRP conjugate (red cap)	1×11mL	2×11mL	2×26mL
Antibody Solution (blue cap)	1×6mL	1×11mL	1×26mL
Concentrated Wash Buffer (20×) (white cap)	1×40mL	1×40mL	1×200mL
Substrate Reagent A (white cap)	1×6mL	1×11mL	1×26mL
Substrate Reagent B (black cap)	1×6mL	1×11mL (brown cap)	1×26mL
Stop Solution (yellow cap)	1×6mL	1×11mL	1×26mL
Positive Control (red cap)	1×1.0mL	1×1.5mL	1×2.0mL
Negative Control (green cap)	1×1.0mL	1×1.5mL	1×2.0mL
Adhesive Membrane	1	2	5
Sealed bag	1	1	2
Instructions	1	1	1

Storage conditions

The kit shall be stored at 2-8 °C. Avoid moisture.

Shelf life: 12 months. Please use within 2 months after opening. The date of manufacture is presented in the label of the box.

Test Apparatus Required but Not Supplied

Microplate reader, adjustable micropipette, constant temperature device (37°C), centrifuge.

Experimental preparation

Restore all reagents and samples to room temperature (adjust to around 25°C) for more than 30 min before use. This is a crucial step to ensure there is no precipitation in the reagents.

1.Sample Preparation:

Serum/plasma: It should be clear, without hemolysis or contamination. Samples can be stored at 2-8°C for up to 1 week, and for long-term storage, they should be kept at -20°C.

2.Solution preparation: Dilute the concentrated wash buffer (20×) by a factor of 20 (Concentrated wash buffer/Deionized water= 1: 19). What we get is the working wash buffer.

ELISA procedure

Place all reagents and samples to room temperature (adjust to around 25°C) for 30min. Gently shake the reagent bottles before use.

Take out the frame of the microplate along with the required number of wells. Then place the unused microplate wells into the sealed bag with the desiccant provided. Store the remaining kit in the refrigerator at 2-8°C.

1.Put the required number of the wells on the plate and set up 2 wells each for negative/positive control.

2.Add 50μL of **negative control** to each negative

control well. Then add 50μL of **positive control** to each positive control well. For each sample well, first add 45 μL of **working wash buffer**, then add 5μL of the sample.

3.Add 50μL of **antibody solution** to each well, shake gently by hand (or use a microplate shaker) for 5s, cover with adhesive membrane and incubate at 37°C (water bath recommended) in the dark for 30 minutes.

4.Discard the liquid from each well. Add 350μL of **working wash buffer** to each well, let stand for 30 seconds, then discard. Repeat the washing process 5 times. Invert the plate and tap it against a thick absorbent paper (or lint-free cloth), with a soft towel placed underneath. (Bubbles that are not removed after tapping dry can be punctured with a clean pipette tip).

5.Add 100μL of **HRP conjugate** to each well, cover with adhesive membrane, and incubate at 37°C in the dark for 30 minutes.

6.Washing. Same as step **4.**

7.First, add 50μL of **substrate reagent A** to each well, followed by 50μL of **substrate reagent B.** shake gently by hand (or use a microplate shaker) for 5s, cover with adhesive membrane, and incubate at 37°C in the dark for 15 minutes.

8.Add 50μL of **stop solution** to each well and shake gently by hand (or use a microplate shaker) for 5s. Read absorbance (**A value**) at 450nm with microplate reader (with 630nm as a reference wavelength). Finish this step within 10min.

Reference Value

Under normal experimental conditions, the A value of the negative control should be ≥0.8, and the A value of the positive control should be ≤ 50% of the A value of the negative control.

Interpretation of Test Results

1. $PI(\text{Percentage Inhibition}\%)=(1-\frac{A_S}{A_{NC}})\times 100\%$,

If PI is ≥50%, it is considered positive; if PI is <50%, it is considered negative.

A_S—the A value of the sample;

A_{NC}—the average A value of negative controls.

2. When the results of this experiment are negative, it indicates that the antibody levels in cattle/sheep is insufficient. It is recommended to administer the corresponding vaccine as a supplementation.

Limitations of the Test Method

This test is only for the qualitative detection of antibodies against FMD-A. Based on the PI Index, a rough assessment of the antibody level as strong, medium, or weak can be made.

Attention

1. During the experiment, gloves and lab coats should be worn. Strict and comprehensive disinfection and isolation protocols should be followed. All experimental waste should be treated as infectious material.

2. The stop solution is corrosive. Avoid contact with skin and clothing. If accidentally contacted, rinse immediately with a large amount of tap water.

3. When taking the microtiter plate out of a refrigerated environment, it should be brought to room temperature before opening the bag. Unused microplate wells should be stored in the sealed bag with a desiccant.

4. During washing, each well should be filled completely with liquid to prevent any residual enzyme on the well's rim from remaining unwashed.

5. The samples used for testing should be kept fresh.

6. The determination of test results must be based on the readings from the microplate reader.

7. Components from different lot numbers must not be mixed.