Foot and Mouth Disease Virus Type A Antibody (FMD-A Ab) 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/plasma of swine, bovine, sheep and goat. It can be employed for the assessment of immunization efficacy.

Principle and Application

This kit comprises a coated Microtiter Plate with FMD-A antibodies, antibody solution, antigen solution, HRP conjugate, and other accompanying reagents. It employs the principle of blocking enzyme-linked immunosorbent assay (blocking ELISA) to detect antibodies against FMD-A in the serum/plasma of swine, bovine, sheep and goat. During the experiment, diluted samples and antigen solutions are added simultaneously to an Microtiter plate for incubation. Antibodies in the sample compete with antibodies on the plate for binding to the antigen, thereby preventing the antigen from binding to the plate. After washing, antibody solution is added for further incubation. Subsequent to another washing step, HRP conjugate is serum/plasma introduced and allowed to incubate. After another washing to remove any unbound HRP conjugate, TMB substrates are added to the wells. This reacts with the enzyme-labeled complex to produce a blue-colored product. The intensity of the color is inversely proportional to the concentration of specific antibodies in the sample. The reaction is then terminated by adding a stop solution, turning the product yellow. The absorbance values in each reaction well are measured at 450nm using a Microtiter Plate reader (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)	1×6mL	1×11mL	1×26mL
Antibody Solution (blue cap)	1×6mL	1×11mL	1×26mL
Antigen Solution (black cap)	1×6mL	1×11mL	1×26mL
Concentrated Wash Buffer (20×) (white cap)	1×40mL	2×40mL	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	1
Dilution plate	1	2	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: The serum/plasma 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 obtained is the working wash buffer.

3.Sample dilution: For bovine, sheep and goat, dilute the prepared sample 32-fold using the working wash buffer (e.g., add 155μL of working wash buffer to the dilution plate, followed by the addition of 5μL of the sample, and mix well). What obtained is the diluted sample.

For pigs, dilute the prepared sample 16-fold using the working wash buffer (e.g., add 75μL of working wash buffer to the dilution plate, followed by the addition of 5 μL of the sample, and mix well). What obtained is the diluted sample.

Negative and positive controls do not require dilution.

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 25 μL of **working wash buffer**, then add 25μL of the **diluted sample**. Finally, add 50μL of **antigen solution** per well.

Note: After adding the antigen solution, the dilution ratio for bovine/ovine/caprine samples is 1:128, and for porcine samples, it is 1:64.

3.Shake the plate gently by hand (or use a microplate shaker) for 10s, cover with adhesive membrane and incubate at 37°C (water bath recommended) in the dark for 30 minutes.

4.Discard the liquid from the wells. Add 350μL of **working wash buffer** per 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 50μL of **antibody solution** 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.Add 50μL of **HRP conjugate** to each well, cover with adhesive membrane, and incubate at 37°C in the dark for 30 minutes.

8.Washing. Same as step **4.**

9.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.

10.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 ≥1.0, 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(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. For Cattle and Sheep: When the results are positive, it indicates the presence of antibodies against FMD-A in the sample, with a titer higher than 1:128, reaching a protective level. Conversely, when the test results are negative, it indicates the absence of antibodies against FMD-A, or the titer is lower than 1:128, not reaching a sufficient protective level.

3. For pigs: When the results are positive, it indicates the presence of antibodies against FMD-A in the sample, with a titer higher than 1:64, reaching a protective level. Conversely, when the test results are negative, it indicates the absence of antibodies against FMD-A, or the titer is lower than 1:64, not reaching a sufficient protective level.

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.

This kit is not suitable for assessing the antibody potency in the immunization of synthetic peptide vaccines.

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.