

**FOOT-AND-MOUTH DISEASE VIRUS (FMDV)
TYPE ASIA - 1 ANTIBODY ELISA KIT
Cat. No. IP100201**

Foot-and-mouth disease virus (FMDV) Type Asia-1 Antibody ELISA Test Kit

1. Usage

The kit is used to detect specific antibody against foot and mouth disease virus (FMDV) Type Asia-1 Antibody in serum of pig, cattle, and sheep qualitatively, for monitoring antibody after FMDV Type Asia-1 immunization.

2. Principle

Foot-and-mouth disease (FMD) is an acute, febrile and highly contagious infectious disease caused by foot-and-mouth disease virus (FMDV), which mainly affects cloven-hoofed animals. Once it occurs, it will cause serious economic losses. FMDV belongs to the foot-and-mouth disease virus genus of the family MicroRNAviridae. There are currently 7 known serotypes, namely O, A, C, SAT1, SAT2, SAT3 and Asia 1.

3. Introduction

This kit uses Indirect ELISA method, the purified FMD virus antigen is pre-coated on enzyme micro-well strips. In the test, add the diluted serum, and after incubation, if the sample contains FMD virus-specific antibodies, it will bind to the antigen on the coated plate, and after washing to remove unbound antibodies and other components; Add the enzyme conjugate to specifically bind to the antigen-antibody complex on the detection plate; then wash to remove the unbound enzyme conjugate; add the substrate to the microwell, and the enzyme catalyzes the formation of a blue product, and add stop solution to terminate the reaction, the absorbance value in the reaction well was measured with a microplate reader at a wavelength of 450 nm.

4. Kit Components

Code	Item	Spec.	Code	Item	Spec.
1	Antigen Coated plates 96 wells	2 plates	7	Positive control	1.6 ml
2	Enzyme Conjugate	11mlx2	8	Negative control	2 ml
3	10X Washing buffer	100 ml	9	Serum dilute plate	2 plates
4	Substrate	22 ml	10	Adhesive Foil	4 pieces
5	Sample diluent	100 ml	11	Instruction	1 piece
6	Stop solution	15 ml			

5. Material required not provided

- 1) Microplate Reader (double-wave length: 450 nm).
- 2) Precise micropipette (single-channel 1-100ul、 0.5-10ul、 multi-channel 30-300ul)
- 3) Constant temperature box or water bath box.
- 4) Oscillator.
- 5) Disposable tips (10ul, 200ul)
- 6) Deionized water

6. Sample Requirement

Take animal whole blood, get serum by using regular method, the serum should be bright and no hemolysis.

7. Preparation

- 1) Bring ELISA reagents to the room temperature ($25\pm 3^{\circ}\text{C}$) for at least 30 min to get best results. Microplate should return to room temperature and dry before opening the package.
- 2) **Washing buffer preparation:** Return 10X Concentrated washing buffer to room temperature before use, if there is salt crystals, shake to dissolve, then dilute it for 10 times with distilled water or deionized water. The diluted washing buffer can be stored at 4°C for about 1 week.
- 3) **Sample dilution:**
Dilute serum at 1:100 on Serum dilute plate.
Note: Both positive control and negative control do not need to be diluted, change tips after taking every sample, mark the sample location on plate accurately, mix every sample evenly before adding to the pre-coated micro-wells.

8. Procedure

- 1) Take the antigen coated plate (the plate can be open and used for several times according to sample quantity each time), add the diluted serum to reaction wells, 100ul/well; meanwhile, set 2 wells for positive control and 2 wells for negative control, take 100ul directly and add into its well, mix gently (do not overflow);
- 2) Cover it with adhesive foil, **incubate at 37°C for 30 minutes;**
- 3) Open the adhesive foil, discard the liquid of the well, add diluted washing buffer to each well, 250ul/well, then discard the liquid, repeat the above step for 4-6 times, at last flap to dry on absorbent paper stack;
- 4) Add Enzyme Conjugate 100ul/well, Cover it with adhesive foil, **incubate at 37°C for 30 minutes;**
- 5) Open the adhesive foil, discard the liquid of the well, wash for 4-6 times as step 4), remember at last, flap to dry on absorbent paper stack;
- 6) Add substrate, 100ul/well, mix it evenly then cover it with adhesive foil, **incubate at 37°C in dark for 10 minutes;**
- 7) Add stop solution 50ul/well to stop the reaction, measure the result in 10 minutes.

9. Results

Read the OD value with ELISA Reader at 450nm (630nm as reference).

For the assay to be valid:

Negative control (N) OD value < 0.2, meanwhile positive control (P) OD value > 0.3;

Calculation method:

$(\text{Sample OD value} - \text{Negative control OD average value}) / (\text{Positive control OD value} - \text{Negative control OD average value}) = \text{IRPC value}$

Results interpretation

IRPC < 0.35: Negative

$0.35 \leq \text{IRPC} < 0.4$: Skeptical

IRPC ≥ 0.4 : Positive

10. Precautions and warnings for users

- 1). Return all reagents to room temperature before use, shake it evenly before use, and store back to 2-8°C after usage.
- 2). Do not use expired products, and do not mix kit components from different batches.
- 3). FMD-Ag coated plates should be sealed and moisture-proof. Put back unused Micro-Well plate into dry foil bag and seal at 2-8 °C.
- 4). Serum dilute plate is disposable, do not repeat use; the max volume of the plate is 300ul/well.
- 5). The instruments used in the experiment (such as: micropipettes, constant humidity incubators, ELISA plate washers, microplate readers) need to be functionally checked before use to ensure their usability and accuracy.
- 6). All reagents are strictly prohibited from contacting skin and mucous membranes. After the inspection, the consumables used shall be harmlessly disposed according to the national regulations.
- 7). Unused microplate strips can be stored in a sealed bag at 2-8°C.
- 8). Each component container in the kit is only used for this component and cannot be reused.

Specifications: 96 wells×2.

Expiry date: On outer-packing of the test kit. (12 months)

Storage: Store at 2~8°C, in the dark, no freezing.