

**FOOT-AND-MOUTH DISEASE VIRUS (FMDV)  
TYPE A ANTIBODY ELISA KIT  
Cat. No. IP100202**

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## Foot-and-mouth disease virus (FMDV) Type A Antibody ELISA Test Kit

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### 1. Usage

The kit is used to detect specific antibody against foot and mouth disease virus (FMDV) Type A Antibody in serum of pig, cattle, and sheep qualitatively, for monitoring antibody after FMDV Type A 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. After FMDV infection or immunization, the main neutralizing epitope of antibodies is in VP1 protein.

### 3. Introduction

The kit use Competitive ELISA method, pure FMDV antigen is pre-coated on enzyme micro-well strips. When testing, add diluted serum sample, after incubation, if there is FMD virus specific antibody, it will combine with the pre-coated antigen, discard the uncombined antibody and other components with washing; then add enzyme labeled anti-FMD virus monoclonal antibody, antibody in sample block the combination of monoclonal antibody and pre-coated antigen; discard the uncombined enzyme conjugate with washing; Add TMB substrate in micro-wells, the blue signal by Enzyme catalysis is in inverse proportion of antibody content in sample, use ELISA reader at 450nm wavelength to measure the absorbance value in reaction wells after adding stop solution to stop the reaction.

### 4. Kit Components

Code	Item	Spec.	Code	Item	Spec.
1	Antigen Coated plates 96 wells	2 plates	7	Stop solution	15 ml
2	Enzyme Conjugate	6 ml x 2	8	Positive control	200ul
3	10XConcentrated Washing buffer	100 ml	9	Negative control	500ul
4	Substrate	22 ml	10	Adhesive Foil	4 pieces
5	Monoclonal antibody working solution	10ml	11	Instruction	1 piece
6	Sample buffer	2.5ml			

## 5. Material required not provided

- 1) Microplate Reader: 96 wells with 450/630nm wavelength.
- 2) Micropipette 10-100ul, 100-1000ul)
- 3) Disposable pipette tips.
- 4) Graduate: 500ul.
- 5) Microplate Washer
- 6) Deionized water

## 6. Sample Requirement

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

## 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 make it dissolved, then dilute it for 10 times with distilled water or deionized water. The diluted washing buffer can be stored at  $4^{\circ}\text{C}$  for about 1 week.

## 8. Procedure

- 1) Set 1 well of positive control and 2 wells of negative controls for each test;
- 2) Add 10ul/well of sample buffer to the antigen coated plate.
- 3) Add 10ul/well of negative control, positive control and serum sample into the well separately, and then add 40ul/well of monoclonal antibody working solution, shake and mix well after adding (do not mix pipette tips);
- 4) Cover it with adhesive foil, **incubate at  $37^{\circ}\text{C}$  for 60 minutes;**
- 5) 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 5 times, at last, flap to dry with the absorbent paper;
- 6) Add Enzyme Conjugate 50ul/well, cover it with adhesive foil, **incubate at  $37^{\circ}\text{C}$  for 30 minutes;**
- 7) Open the adhesive foil, discard the liquid of the well, wash for 5 times as step 5), remember at last, flap to dry on an absorbent paper stack;
- 8) Add substrate, 100ul/well, mix it evenly then cover it with adhesive foil, **incubate at  $37^{\circ}\text{C}$  in dark for 10 minutes;**
- 9) Add stop solution 50ul/well to stop the reaction, measure the result in 10 minutes.

## 9. Result

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

### For the assay to be valid:

Negative control (N) OD value > 0.5, meanwhile positive control (P) blocking rate > 50%;

### Calculation method:

PI (blocking rate) =  $\{1 - (\text{sample OD value} \div \text{negative control OD average value})\} \times 100\%$

### Results interpretation

PI (blocking rate) > 45% is judged as positive;

PI ≤ 45% is judged as negative

### The corresponding relationship between the antibody titer detected by liquid-phase blocking ELISA and the PI value detected by this kit:

Liquid blocking antibody titer	<1:64	1:64-1:128	1:128-1:256	>1:256
PI	<45%	45%-60%	60%-80%	>80%

**Note:** Individual samples may not strictly conform to this correspondence due to inter-individual differences.

## 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.