

**FOOT-AND-MOUTH DISEASE VIRUS
(FMDV) NON-STRUCTURAL PROTEIN
ANTIBODY ELISA KIT
MANUAL**

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**Foot-And-Mouth Disease Virus (FMDV) Non-Structural Protein Antibody 3ABC
ELISA Test Kit**

Catalogue Number. IP100203

Principle

Foot-and-mouth disease virus (FMDV) Non-structural protein Antibody 3ABC ELISA Test Kit is suitable for test serum of cattle, sheep, goats and pigs, it can distinguish between immunized animals and wild-infected animals. This kit uses block ELISA method, FMDV-3ABC 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 A value in reaction wells after adding stop solution to stop the reaction.

Specifications: 96 wells × 2.

Components

	Code item	Spec.
1	FMDV-3ABC Ag Coated plates 96 wells	2 plates
2	Enzyme Conjugate	11mlx2
3	10X Concentrated Washing buffer	100 ml
4	Substrate	22 ml
5	Sample diluent	100 ml
6	Stop solution	15 ml
7	Negative control	2 ml
8	Positive control	1 ml
9	Adhesive Foil	4 pieces
10	Instruction	1 piece

Material required not provided

1. Micropipettes: 0.5µL~10µL、10µL~100µL、100µL~1000µL
2. Disposable pipette tips
3. Measuring cylinder: 500 ml
4. 96 wells microplate reader
5. Distilled water or deionized water
6. Bottle or microplate washing machine

Sample preparation

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

Washing buffer preparation

Return 10X Concentrated washing buffer into room temperature before use, if there is salt crystals, shake to make it dissolved, then dilute it at 10 times with distilled water or deionized water. The diluted washing buffer can store at 4°C for about 1 week.

Operation procedures

1. Take the antigen coated plate (the plate can be open and used for several times according to sample quantity each time), for every test, set 1 well for positive control and 2 wells for negative control, positive control and negative control do not need dilute, take 100ul directly and add into its well;
2. Add Sample diluent to reaction wells, 80ul/well, then add serum sample to the reaction wells, 20ul/well, blow and mix evenly (Do not mix use tips)
3. Cover it with Adhesive Foil, react at 22 ($\pm 3^\circ\text{C}$) for Overnight (about 16-18 hours);
4. 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 6 times, at last flap to dry with the absorbent paper;
5. Adding Enzyme Conjugate 100ul/well, Cover it with Adhesive Foil, incubate at 22 ($\pm 3^\circ\text{C}$) for 60 minutes;
6. Open the adhesive foil, discard the liquid of the well, washing for 6 times as step 4), remember at last flap to dry with the absorbent paper;
7. Add substrate, 100ul/well, mix it evenly then cover it with Adhesive Foil, incubate at 22 ($\pm 3^\circ\text{C}$) in dark for 15 minutes;
8. Add stop solution 50ul/well to stop the reaction, measure the result in 10 minutes.

Results judgment

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

For the assay to be valid:

Negative control (N) OD value > 0.6 , meanwhile positive control (PI) blocking rate $> 50\%$;

Calculate method:

$\text{PI}(\text{blocking rate}) = 1 - (\text{Sample OD value} / \text{Average OD value of Negative control})$

Results interpretation

$\text{PI}(\text{blocking rate}) > 50\%$: Positive

$\text{PI}(\text{blocking rate}) \leq 50\%$: Negative

Notes

1. Return all reagents into room temperature before use, put the reagents at room temperature for at least 1 hour. Shake it evenly before use, and store back to 2-8°C after usage.

2. Do not mix use reagents from different kits and different lot no., prevent the reagents been polluted when using.
3. Substrate and stop solution may have irritation to skin and eyes, be careful to use.
4. Do not expose Substrate to strong light and avoid contact with the oxidant.
5. FMDV-3ABC Ag coated plates should be sealed and moisture-proof. Put back unused Micro-Well plate into dry foil bag and sealed at 2-8 °C.
6. All wastes should be treated well to avoid pollution before discarding.
7. Strict compliance with the operating instructions can get the best results. Pipetting operation, timing, and washing of the whole process must be precise.
8. FMDV-3ABC Ag Coated plates is disposable, do not repeat use.

storage: store at 2-8°C, dark, sealed, dry place, no frozen.

Expiry date: 12 months; date of production is on box.