

**FOOT-AND-MOUTH DISEASE VIRUS
(FMDV) TYPE O ANTIBODY ELISA KIT
MANUAL**

Foot-and-Mouth Disease Virus (FMDV) Type O Antibody ELISA Test Kit

Catalogue Number. IP100200

Principle

This kit is used to detect specific antibody against foot and mouth disease virus (FMDV) Type O Antibody in serum of bovine, goat and porcine qualitatively, for monitoring antibody after FMDV Type O immune.

This kit use blocking ELISA method, 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-VP1 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	Antigen Coated plates 96 wells	2 plates
2	Enzyme Conjugate	22 ml
3	10Xconcentrated Washing buffer	100 ml
4	Substrate	22 ml
5	Sample dilution	100 ml
6	Stop solution	11 ml
7	Positive control	1 ml
8	Negative control	2 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 be bright and no hemolysis

Washing buffer preparation

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

Sample dilution

At serum dilution plate, dilute serum at 1:100 with sample diluent.

Notice: Negative control and Positive control do not need dilution. Exchange tip after taking sample every time, record the situation of the sample on plate accurately. Shake the sample evenly before adding it

Operation procedures

1. Adding sample: Take out the required coated plates according to sample quantity (Can be detached) and record the sample position on a worksheet. For single-wave length test, set one blank control well, add nothing; for double-wave length test, do not set blank control well. Set 2 wells for negative control serum and 2 wells for positive control serum, add undiluted negative and positive control serum to its well accordingly, 100 μ L/well. Others are wells for samples, add 100 μ L/well of Sample diluent solution, then add 1 μ L serum sample separately (there will be color change after adding sample).
2. Mix gently for 30s, incubate at 37°C for 30 min.
3. Remove adhesive foil. Pour the liquid out of the wells, add Washing solution(dilute the Wash Concentrate at 20 times with deionized water) into each well fully, stand for 1 min. Repeat 5 times, at last time pat to dry on absorbent paper.
4. Add 100 μ L enzyme conjugate into each well (except the blank well, also do not add any liquid to blank well).
5. Cover plate with new adhesive foil. Incubate at 37 °C for 30 min.
6. Repeat step 3(washing).
7. Add 50 μ L substrate A and substrate 50 μ L B into each well, mix properly, incubate for 15 min at 37 °C in the dark with new adhesive foil.
8. Add 50 μ L stop solution into each well, mix gently and determine the result within 5-30 min.
9. For single-wavelength test, measure the A value with a photometer at 450 nm, set zero for the blank well, and read A value of each well; For double-wavelength test, measure the A value with a photometer at 450 nm (Reference-wavelength: 630 nm), read A value of each well.

Results

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

For the assay to be valid:

OD Value of Negative control (N)< 0.2, meanwhile average OD value of Positive control (P)>0.4.

Calculation method:

Sample OD value / Positive control OD Average value= S/P value

The result is judged by S/P value:

If $S/P \geq 0.5$, it is positive; If $S/P < 0.5$, it is 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) FMD-Ag coated plates should be sealed and moisture-proof. Put back unused MicroWell 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) FMD-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.