VERSION 1.01

FOOT-AND-MOUTH DISEASE VIRUS (FMDV) TYPE A ANTIBODY ELISA KIT

MANUAL

ELISAKITS.ONLINE

By Immunomart

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

Catalogue Number. IP100202

Principle

This kit is used to detect specific antibody against foot and mouth disease virus (FMDV) Type A Antibody in bovine and goat serum qualitatively, for monitoring antibody after FMDV Type A immune.This kit use indirect ELISA method, pured 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 labled 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 wavelenth 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	1/2 plate
2	Enzyme Conjugate	6/11 ml
3	10X Washing buffer	50/100 ml
4	Substrate	11/22 ml
5	Sample diluent	50/100 ml
6	Stop solution	6/11 ml
7	Positive control	0.8/1.6 ml
8	Negative control	0.8/1.6 ml
9	Serum dilute plate	1/2 plate
10	Adhesive Foil	2/4 pieces
11	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℃ 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 dilute. 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 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, both positive control and negative control do not need dilute, take 100ul directly and add into its well, mix gently(do not overflow);
2.Cover it with Adhesive Foil, incubate at 37 ℃ 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 with the absorbent paper;
4. Adding Enzyme Conjugate50ul/well, Cover it with Adhesive Foil, incubate at 37 ℃ for 30 minutes;
5.Open the adhesive foil, discard the liquid of the well, washing for 4-6 times as step 4), remember at last flap to dry with the absorbent paper;
6. Add substrate: add substrate, 100ul/well, mix it evenly then cover it with Adhesive Foil, incubate at 37 ℃ in dark for 10 minutes;
7.Add stop solution 50ul/well to stop the reaction, measure the result in 10 minutes.

Results judgement

Read the OD value with ELISA Reader at 450nm (630nm as reference).
For the assay to be valid:
Negative control (N) OD value< 0.15, meanwhile positive control (P) OD value > 0.3;

Calculate method:

(Sample OD value - Negative control OD average value)/(Positive control OD value - Negative control OD average value) = IRPC value

Results interpretation

IRPC<0.3: Negative
0.3≤IRPC<0.4: Skeptical
IRPC≥0.4: Positive

Notes

Return all reagents into room temperature before use, shake it evenly before use, and store back to 2-8℃ 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 ℃.
6) All wastes should be treated well to avoid pullution 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) Serum dilute plate is disposable, do not repeat use; the max volume of the plate is 300ul/well.

storage: store at 2-8℃, dark, sealed, dry place, no frozen..
Expiry date: 12 months; date of production is on box.