FMD Serotype A antibody ELISA Kit

FMD A Ab Test

Product Number: E30031

Product Unit: 1 plate, 96T / 2 plates, 192T / 5plates, 480T

Manufacturer Information

Ring Biotechnology Co., Ltd

E-mail: export@ringbio.com diego@nbgen.com Web: www.ringbio.com

Add: Building 3, Zhongtongtai TechnoPark, No. 11, Kechuang 14th St, Beijing 100176, CHINA

Tel: +86-10-56267496 Technical Support & Service: +86-13811393460

1. Introduction

The foot-and-mouth disease virus (FMDV) is the pathogen that causes foot-and-mouth disease. The disease, which causes vesicles (blisters) in the mouth and feet of bovids, suids, ovids, caprids and other cloven-hoofed animals is highly infectious and a major plague of animal farming, which causes billions of damage to the animal farming industry before the vaccine was developed.

2. Description of Test

The FMD serotype A ab ELISA kit is used to measure the amount of antibodies generated by vaccination or infection of FMDV serotype A. A FMDV serotype A antigen has been pre-coated in microplates. Samples are added to the microplate wells where any specific antibodies present will bind and form antigen-antibody complexes. Non-specific antibodies are discarded by a washing step. When the monoclonal antibody conjugate (ready to use) specific to FMDV serotype Asian I antigen is added, it will bind to the antigen only if there are no antibodies in the sample blocking the antigen (negative animals). In case there were antibodies blocking the antigen (infected animals), the conjugate would not be able to bind it. The binding is detected by the development of a colorimetric reaction after the addition of the substrate.
This ELISA kit can be used to detect FMD A antibody in **swine / bovine / caprine / ovine serum (or plasma)**.

3. Precautions

- Store the kit at 2-8℃,Check the lot number and expiration date before use.
- Bring the test kit to room temperature before use. For example, take it out from the cold storage and put at room temperature for at least 30min.
- The stop solution in the kit is acidic, please make sure do not touch it with your hand or skin.
- The component of the kit is noninfectious, but the field sample shall be treated as potentially infectious. Please handle all these materials properly according to your lab regulations.
- After experiment, all lab materials shall be handled properly according to local regulations.

4. Limitations of Test

This ELISA kit is currently designed for research or *in vitro* veterinary use. We recommend validating in your own lab with different methodologies to confirm the performance. If it is not used for the mentioned purpose, please contact us for help.

5. Reagent Provided

The kit contains the following items.

Item No.	Description	Quantity
1	Microplate pre-coated with FMD A antigen	5 plates
2	Positive Control	1 X 2 ml
3	Negative Control	1 X 2 ml

4	Sample buffer	2 X 60 ml
5	HRP enzyme conjugate	1 X 60 ml
6	TMB substrate	1 X 60 ml
7	Stop solution -- Not provided	1 X 60 ml
8	25X Wash buffer	1 X 60 ml
9	Kit instruction	1set

Note: Stop solution is 2M sulfuric acid, which is not provided due to air cargo shipment, which shall be prepared by client.

6. Instrument Required

- ELISA reader
- Micropipette 20-200ul
- Micropipette Multi-Channel 50-300ul

7. Reagent Preparation

- **Wash buffer**: dilute the 25xWash buffer provided in the kit with deionized water in the volume ratio of 1:24. For example, 1ml 25xWash buffer + 24ml deionized water
  The diluted wash buffer can be stored at room temperature for 2 weeks.
  No other reagent is required. Please remember to return all kit component to room temperature before use. For example, keep the components at RT for 60min.

8. Sample Preparation

- **Sample:** dilute with sample buffer in the volume ratio of 1:39. **For example**: 5ul sample + 195ul sample buffer.
  Please freshly prepare before assay. Change a new micropipette tip for different sample.

9. Assay Procedure

1) Make sure the kit and all test samples are returned to room temperature before use. Shake each reagent gently before adding into the well.
2) Open the kit, read the kit instruction carefully to make sure all technical points are understood clearly.
3) Take the microplate from the zip-bag, and take needed microwells, store the rest into the zip-bag. Make marks of the plate layout. **Running the test in duplicated wells** is recommended to minimize operational error.
4) **Add Positive control**: add **50ul positive control** into the wells.
5) **Add Negative control**: add **50ul negative control** into the wells.
6) **Add Sample**: add **50ul sample** into the other wells.
7) Cover the plate and then incubate at **room temperature for 60min**.
8) **Washing**: pour the liquid out from the wells and wash with wash buffer (300ul per well) for 3 times. Tap the residue liquid against absorbent paper to make sure the plate is dry after washing.
9) **Add enzyme conjugate**: add 50ul of HRP conjugate into each well. Cover the plate again and then incubate at **room temperature for 60min**.
10) **Washing**: repeat the wash step again.
11) **Add substrate**: add TMB substrate into each well, 100ul per well. Cover the plate again and then incubate at **room temperature for 10min**. Color reaction will occur in the plate.

12) **Stop the reaction**: add 50ul stop solution into each well, the color will turn yellow from blue.

13) **Read the plate**: using ELISA reader to read the plate at 450nm.

10. Result Determination

10.1 Criteria of a valid assay

Mean OD of Negative Control > 0.5

Mean OD of Positive Control < 1.0

10.2 Criteria of Positive and Negative results.

1) Calculation of **Inhibition Rate**:

$$\frac{\textbf{Mean OD of Negative Control – Mean OD of Sample}}{\textbf{Mean OD of Negative Control}} * 100\ \% = \textbf{Inhibition Rate \%}$$

2) Criteria of Positive and Negative results.

Positive: Blocking Rate **≥ 50%**

Negative: Blocking Rate **< 50%**

11. Storage and expiration

The kit shall be store at 2-8°C, avoid direct sunlight.

The valid period is 12 months.

12. References

LN Ma, J Zhang, HT Chen, at al, An overview on ELISA techniques for FMD, Virol J. 2011; 8: 419.