Foot and Mouth Disease Virus Type A Antibodies ELISA Kit

Catalog No: E-AD-E124

96T/96T*3/96T*5

Version Number:	V1.1
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This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Toll-free: 1-888-852-8623 Tel: 1-832-243-6086 Fax: 1-832-243-6017

Email: techsupport@elabscience.com

Website: www.vetassay-elab.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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Test principle

This ELISA kit is consisted of Micro-plate pre-coated with FMDV-A antibody, Antibody Working Solution, HRP Conjugate, Antigen Solution and other reagents. It applies Inhibition-ELISA as the method for the detection of FMDV-A antibody in Cattle, Goat, Sheep, Porcine samples. Diluted samples and antigen are added into the Micro-plate and incubate. Antibodies in sample will compete with antibody pre-coated on the Micro-plate for the antigen and block the combination between the antigen and the Micro-plate. Then wash and add the Antibody Working Solution to incubate. Wash and add HRP Conjugate, incubate. Then wash away the unbound HRP Conjugate. Add TMB substrate to the wells, it will react with the enzyme and become blue, the shade of color is of negative correlation with the content of specific antibody in the samples. At last, end the reaction by adding Stop Solution to produce a yellow product. Measure the absorbance value of each well by using a Micro-plate Reader with 450 nm wavelength, then the presence of FMDV-A antibody can be determined.

Kit components

Item	Specifications
ELISA Microtiter plate	96 wells
HRP Conjugate	6 mL
10×Concentrated Wash Buffer	100 mL
Substrate Reagent	11 mL
Antibody Working Solution	5 mL
Sample Diluent	3 mL
Stop Solution	15 mL
Positive Control	0.2 mL
Negative Control	0.5 mL
Plate Sealer	1 piece
Sealed Bag	1 piece
Manual	1 copy

Experimental instrument

Microplate Reader with 450nm wavelength filter or dual-wavelength (450/630nm)

High-precision transferpettor, EP tubes and disposable pipette tips

37℃ incubator or water bath

Deionized or distilled water

Absorbent paper

Notes

1. Wear gloves and work clothes during experiment, and the disinfection and isolation system should be strictly performed. All the waste should be handled as contaminant.
2. The stop solution is corrosive, it should be avoided to contact with skin and clothing. Wash immediately with plenty of water if contacted carelessly.
3. FOR RESEARCH USE ONLY. ELISA Microtiter plate should be covered by plate sealer. Avoid the kit to strong light.
4. Concentrated wash buffer at low temperature condition is easy to crystallize, it should be adjusted to room temperature in order to dissolve completely before use.
5. Each well must be filled with liquid when washing in order to prevent residual free enzyme.
6. The tested sample should keep fresh.
7. The results shall depend on the readings of the microplate reader.
8. **Each reagent is optimized for use in the E-AD-E124. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-AD-E124 with different lot numbers.**
9. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.

Storage and expiry date

Store at 2-8℃. Avoid freeze.

Please store the opened plate at 2-8℃, the shelf life of the opened kit is up to 1 month.

Expiry date: expiration date is on the packing box.

Sample pretreatment

1. **Serum/plasma:** Use the conventional method to prepare serum/plasma, the serum/plasma must be clear, no hemolysis and no pollution. Samples can be stored at 2-8℃ for one week, and it should be stored at -20℃ for a long term storage.
2. **Wash Buffer:** The **10×Concentrated Wash Buffer** should be adjusted to room temperature to make the sediment dissolved fully before use, then dilute it with deionized water at 1 : 9.

Assay procedure

Restore all reagents and samples to room temperature before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2-8℃.

1. **Number:** number the sample and control in order, and keep a record of control wells and sample wells. Take out the microtiter plate, set 1 well for positive control and 2 wells for negative control. **Samples need test in duplicate**.
2. **Add sample diluent:** add 10 μL **Sample Diluent** into each well.
3. **Add sample:** add 10 μL of **positive/negative control** and **Sample** into each well.
4. **Antibody Working Solution:** add 40 μL **Antibody Working Solution** into each well, cover the plate sealer and incubate for 60 min at 37℃ in shading light.
5. **Wash:** Remove the liquid in each well. Immediately add 250 μL of wash buffer to each well and wash. Repeat wash procedure for 4-6 times, 30 s intervals/time. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
6. **HRP conjugate:** add 50 μL of **HRP Conjugate** into each well, cover the plate sealer and incubate for 30 min at 37℃ in shading light.
7. **Wash:** Repeat Step 5 for washing.
8. **Color Development:** Add 100 μL of **Substrate Reagent** into each well, mix thoroughly. Incubate for 10 min at 37℃ in shading light.
9. **Stop reaction:** add 50 μL of **Stop Solution** into each well, mix thoroughly.
10. **OD Measurement:** Measure the OD-value of each well by using a microplate reader with 450 nm wavelength (use 630 nm as reference wavelength). This step should be finished in 10 min after stop reaction.

Reference value

Normally, OD value of negative control (N) > 0.5 and PI of positive control > 45%.

Analysis of results

1. PI (Percentage of inhibition) = (1- (OD_{sample} / Average $OD_{negative}$)) ×100%.
2. Positive result : PI > 45%
3. Negative result : PI ≤ 45%
4. The positive result of this detection suggests that the sample contains FMDV-A antibody, and the titer is more than 1:64, which has reached the protective level. The negative result of this detection suggests that there is no FMDV-A antibody in the sample, or the titer is less than 1:64, which has not reached the protective level.
5. To know the final titer of antibody in sample, dilute the sample with gradient dilution. The highest dilution ratio which making the PI > 45% is the final titer of antibody in this sample.

Limitations

1. This kit can only be used to qualitatively detect the FMDV-A antibody in serum of Cattle, Goat, Sheep, Porcine. A rough estimate (high, general, low) of the antibody concentration can be calculated according to the PI value.
2. The detection results of this kit are only for reference. For confirmation of the result, please combine the symptoms and other methods of detection, this detection cannot be used as the only criteria for result.

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