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

Catalog No.: abx157259

Size: 96 tests

Storage: Store all components at 4°C for up to 6 months.

Application: For detection and quantification of FMDV-A-Ab content in cattle, goat, sheep and swine serum.

Principle of the Assay: This kit is based on sandwich enzyme-linked immuno-sorbent assay technology. An antigen is pre-coated onto a 96-well plate. Standards, test samples, and biotin-conjugated reagent are added to the wells and incubated. The HRP-conjugated reagent is then added, and the whole plate is incubated. Unbound conjugates are removed using wash buffer at each stage. TMB substrate is used to quantify the HRP enzymatic reaction. After TMB substrate is added, only wells that contain sufficient FMDV-A-Ab will produce a blue coloured product, which then changes to yellow after adding the acidic stop solution. The intensity of the yellow colour is proportional to the FMDV-A-Ab amount bound on the plate. The Optical Density (OD) is measured spectrophotometrically at 450 nm in a microplate reader, from which the concentration of FMDV-A-Ab can be calculated.

Kit components

1. 96-well microplate
2. Wash Buffer: (10X) 100 ml
3. Detection Reagent A: 5 ml
4. Detection Reagent B: 6 ml
5. TMB Substrate: 11 ml
6. Stop Solution: 15 ml
7. Positive control: 200 µl
8. Negative control: 500 µl
9. Plate sealer: 2
10. Hermetic bag

Materials Required But Not Provided

1. Microplate reader (450 nm)
2. Distilled/deionized water
3. Pipette and pipette tips
4. Vials/tubes
5. 37°C incubator/water bath
6. Centrifuge
7. Vortex mixer

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation method are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 1 hr. Centrifuge at approximately 2000 × g for 15 mins at 4°C. If a precipitate appears, centrifuge again. Take the supernatant, keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.

Samples should not contain detergents such as SDS Tween-20, NP-40 and Triton X-100, or reducing agents such as DTT.

We recommend carrying out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.

- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. Reagents

- **Wash buffer**: Bring to room temperature, and dilute the 10X Wash buffer 1:9 with deionized water (for example, add 100 ml of 10X Wash buffer to 900 ml deionized water). Mix fully.

B. Assay Procedure

1. Set the sample, positive control and negative control wells and record their positions. Each sample should be tested in duplicate, and each assay should use at least 1 positive control well, and 2 negative control wells.
2. Add 10 µl of positive control to the positive control well.
3. Add 10 µl of negative control to the negative control wells.
4. Add 10 µl of sample to each sample well.
5. Add 40 µl of Detection Reagent A to all wells, and mix fully.
6. Cover the plate and tap to mix. Incubate at 37°C in the dark for 60 minutes.
7. Remove the cover and discard the solution. Wash the plate 3 times with 1X Wash Buffer. *Fill each well completely with Wash buffer (350 µl) using a multi-channel Pipette or autowasher (1-2 mins soaking period is recommended). Complete removal of liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean absorbent paper towels.*
8. Add 50 µl of Detection Reagent B to all wells, and mix fully.
9. Cover the plate and tap to mix. Incubate at 37°C in the dark for 60 minutes.

10. Remove the cover, discard the solution and repeat the wash process as described above, 5 times.
11. Aliquot 100 µl of TMB Substrate into each well. Cover the plate with the plate sealer. Gently tap the plate to mix thoroughly. Incubate at 37°C for 10-20 minutes. The incubation time is for reference only, the optimal time should be determined by end user. Avoid exposure to light.
12. Aliquot 50 µl of Stop Solution into each well. It is important that the Stop Solution is mixed quickly and uniformly throughout the microplate to inactivate the enzyme completely.
13. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the OD at 450 nm immediately.

Calculations

$$\text{Inhibition (\%)} = 1 - \frac{\text{OD}_{\text{Sample}}}{\text{OD}_{\text{Negative control}}} \times 100$$

where:

$\text{OD}_{\text{Sample}}$	Absorbance of the sample
$\text{OD}_{\text{Negative control}}$	Average absorbance of the negative controls

Interpretation of results

The OD value of the negative control should be > 0.5, and the Inhibition (%) of the positive control should be > 50%, otherwise the test is invalid.

- If the Inhibition (%) of a sample is > 45%, the sample should be considered positive for FMDV-A-Ab.
- If the Inhibition (%) of a sample is ≤ 45%, the sample should be considered negative for FMDV-A-Ab.
- If animals are not immunized, a positive result may indicate infection with FMDV-A. This result should not be used alone for diagnosis, and should be used with other detection methods.