Cattle and Goat Foot and Mouth Disease Virus Type O Antibody ELISA Kit

Catalog No.: abx364926

Size: 96 tests

Detection Range: Qualitative

Sensitivity: Qualitative

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

Application: The qualitative detection of Foot and Mouth Disease Virus Type O Antibody in Cow, Goat, and Sheep serum and plasma.

Principle of the Assay: This kit is based on competitive enzyme-linked immuno-sorbent assay technology. An antigen is pre-coated onto a 96-well plate. Controls, test samples and HRP-conjugated reagent are added to the wells and incubated. If FMDV-O antibodies are present in the sample, they will compete with antibodies in the Working Antibody Solution to bind the antigen pre-coated onto the plate. Unbound conjugates are removed using wash buffer. TMB substrate is used to quantify the HRP enzymatic reaction. After TMB substrate is added, only wells that contain sufficient Foot and Mouth Disease Virus Type O Antibody will produce a blue colored product, which then changes to yellow after adding the acidic stop solution. The intensity of the yellow color is inversely proportional to the Foot and Mouth Disease Virus Type O Antibody present in the sample. The Optical Density (OD) is measured spectrophotometrically at 450 nm in a microplate reader, from which the presence of Foot and Mouth Disease Virus Type O Antibody can be determined.

Kit components

1. Pre-coated 96-Well Microplate: 12 x 8
2. Wash Buffer (20X): 40 ml
3. Working Antibody Solution: 6 ml
4. Detection Antibody Solution: 11 ml
5. Positive Control: 1 ml
6. Negative Control: 1 ml
7. Substrate Reagent A: 6 ml
8. Substrate Reagent B: 6 ml
9. Stop Solution: 6 ml
10. Plate Sealer: 3
11. Hermetic bag: 1

Materials Required But Not Provided

1. 37°C incubator
2. Multi and single channel pipettes and sterile pipette tips
3. Squirt bottle or automated microplate washer
4. Distilled water
5. Absorbent filter papers
6. Microplate reader (450 nm, or 450/630 nm)
7. ELISA Shaker

Protocol

A. Preparation of samples and reagents

1. Samples

Analyze immediately or store samples at 2 – 8°C (within 24 hours). For long term storage, aliquot and store at -20°C or -80°C. Avoid multiple freeze-thaw cycles.

- **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 hour. Centrifuge at approximately 1000 × g for 20 mins. If precipitate appears, centrifuge again. Assay immediately or aliquot and store at -20°C or -80°C.
- **Plasma**: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 mins at 1000 x g, within 30 mins of collection. If precipitate appears, centrifuge again. Avoid hemolytic samples.

Note:

- Store frozen samples undiluted. Once ready to analyze, thaw samples and dilute.
- Fresh samples, or recently obtained samples, are recommended to prevent protein degradation and denaturation that may lead to erroneous results.
- NaN_3 cannot be used as a test sample preservative, since it inhibits HRP.
- If possible, prepare solid samples using sonication and/or homogenization, as lysis buffers may (on occasion) interfere with the kit's performance.
- If a sample is not indicated in the manuals applications, a preliminary experiment to determine the suitability of the kit will be required.

2. Reagents

- **Wash Buffer**: Dilute the concentrated Wash buffer 20-fold (1/20) with distilled water (i.e. add 40 ml of concentrated wash buffer into 760 ml of distilled water). If crystals have formed in the concentrated Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

B. Assay Procedure

Equilibrate the kit components and samples to room temperature prior to use. *It is strongly recommended to measure samples in duplicate*.

1. Set 2 positive control, 2 negative control, and the desired test sample wells on the pre-coated plate respectively, and record their positions. For best results, when adding solution to the wells, add it to the bottom of each well without touching the side walls. Pipette the standards and samples up and down to mix before adding to the wells. Avoid foaming or bubbles.
2. Aliquot 50 µl of negative and 50 µl positive control into the corresponding control wells. Aliquot 5 µl of sample and 45 µl of diluted Wash Buffer to the sample wells.
3. Add 50 µl of Working Antibody Solution to each well. Cover with a plate sealer, and tap the plate gently to mix. Incubate at 37°C for 30 minutes.
4. Remove the liquid in each well, and immediately add 300 µl of Wash Buffer to each well, using a multi-channel pipette or autowasher. A 1 – 2 minute soaking period is recommended. Repeat the wash procedure 5 times, at 30 second intervals. Remove any remaining Wash Buffer by aspirating or decanting, invert the plate and blot it onto clean absorbent filter paper.

5. Add 100 µl of Detection Antibody Solution to each well. Cover with a plate sealer, and tap the plate gently to mix. Incubate at 37°C for 30 minutes.
6. Wash the plate by repeating Step 4.
7. Add 50 µl of Substrate Reagent A and 50 µl of Substrate Reagent B to each well. Cover with a plate sealer, and use a microplate shaker to mix fully. Incubate at 37°C for 15 minutes.
8. Add 50 µl of Stop Solution to each well, and mix thoroughly.
9. 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.

Data Analysis:

- Mean OD of the Negative Control should be ≥ 0.8.
  Mean OD of the Positive Control should be ≤ 50% of the mean OD of the Negative Control.

- Percentage of inhibition (PI) = (1 – OD of sample / mean OD of negative control) x 100%

PI ≥ 50% is considered a **positive result**, indicating FMDV-O-Ab is present in the sample;
PI < 50% is considered a **negative result**.

Precautions:

- Before using the kit, centrifuge the tubes to bring down the contents trapped in the lid.
- Do not leave the wells uncovered for extended periods between incubations. The addition of reagents for each step should not exceed 10 minutes.
- Ensure that the plate is properly sealed or covered during the incubation steps, and that the time and temperature are controlled. Do not reuse pipette tips and tubes.
- Do not use expired components, or components from a different kit.
- The Detection and Substrate Reagents should be used under sterile conditions, and light exposure should be minimized. Do not discard any residual solution back into the vial.
- Please note that this kit is optimized for detection of native samples, rather than recombinant proteins or synthetic chemicals. We are unable to guarantee detection of recombinant proteins, as they may have different sequences or tertiary structures to the native protein.