Foot and Mouth Disease Virus NSP Antibody ELISA Kit

Catalog No.: abx055782

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 NSP Antibody in Pig, Cow, Goat, and Sheep serum.

Principle of the Assay: This kit is based on 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. 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 NSP 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 proportional to the Foot and Mouth Disease Virus NSP Antibody ELISA Kit amount bound on the plate. The Optical Density (OD) is measured spectrophotometrically at 450 nm in a microplate reader, from which the presence of Foot and Mouth Disease Virus NSP Antibody can be determined.

Kit components

1. Pre-coated 96-Well Microplate: 12 x 8
2. Wash Buffer: (10X) 100 ml
3. Positive Control: 1 ml
4. Negative Control: 2 ml
5. Sample Diluent Buffer: 100 ml
6. Detection Reagent: 11 ml
7. TMB Substrate: 11 ml
8. Stop Solution: 15 ml
9. Plate Sealer: 3
10. 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. 1.5 ml tubes
5. Distilled water
6. Absorbent filter papers
7. 100 ml and 1 liter graduated cylinders
8. Microplate reader (wavelength: 450 nm, or 450/630 nm)
9. 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 hr. 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.

Note:

- **Analyze samples at a 5-fold (1/5) dilution** (e.g. add 20 µl of undiluted sample to 80 µl of Sample Diluent Buffer to obtain 100 µl of diluted sample).
- 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.
- NaN3 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 10-fold (1/10) with distilled water (i.e. add 100 ml of concentrated wash buffer into 900 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 recommended to measure in duplicate.

1. Set 2 positive and 2 negative control, test sample and control (zero) wells on the pre-coated plate respectively, and record their positions. Add the solution 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 100 µl of negative and 100 µl positive control into the set wells. Aliquot 100 µl of sample diluent buffer in the control (zero) blank well.
3. Aliquot 100 µl of appropriately diluted sample (1/5 dilution) into the test sample wells. Gently tap the plate to mix, or use a microplate shaker.
4. Cover the plate with a plate sealer and incubate overnight at 22°C (16-18 hours).
5. Remove the cover and discard the liquid. Wash the plate 5 times with 1X Wash Buffer. Fill each well completely with Wash buffer (250 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.

6. Aliquot 100 µl of Detection Reagent to each well (except the blank well). Cover the plate with a plate sealer and incubate for 60 minutes at 22°C.
7. Remove the cover, discard the liquid, and repeat the wash process as described above, 5 times.
8. Aliquot 100 µl of TMB Substrate into each well. Cover the plate with the plate sealer and gently tap the plate to mix thoroughly. Incubate at 22°C for 15 mins. The incubation time is for reference only, the optimal time should be determined by end user. Avoid exposure to light.
9. 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.
10. 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 > 0.6. The percentage of inhibition (PI) of the positive control should be >50%.
- Percentage of inhibition (PI) = (1-OD of sample / mean OD of negative control) x 100%

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 TMB substrate should be used under sterile conditions, and light exposure should be minimized. Unused substrate should be colorless, or a very light yellow in appearance. 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.