Foot and Mouth Disease Virus Type O Antibody ELISA

For veterinary use only

BIONOTE FMD Type O Ab ELISA

1. Explanation of the Test

The BIONOTE FMD Type O Ab ELISA is a Competitive Enzyme Linked Immunosorbent Assay for the qualitative detection of antibody (Ab) against FMDV Type O structural protein (SP) in serum or plasma of cattle, pigs, and goats.
For testing, ELISA plates are incubated with a mixture of sample and monoclonal antibody-HRP for 90 minutes at 37 ℃ temperature. During the first incubation, if there are FMDV Type O SP Abs in the test sample, the antibodies and HRP conjugated monoclonal antibodies against FMDV Type O SP competitively bind to the antigens on the well. After incubation, all unbounded materials are removed at the washing step. The enzyme, linked to the complex, is revealed by the addition of a substrate. The enzyme activity will thus be directly inversely proportional to the FMDV Type O SP Abs in a sample. The reaction is stopped by adding a stop solution, and colorimetric reading will be performed by using a spectrophotometer at 450 nm and reference wavelength at 620 nm.
The highly specific selected recombinant FMDV Type O SP antigens are used as capture material in this test. These enable the FMD Type O Ab ELISA to identify FMDV Type O SP Abs in a sample, with a high degree of accuracy.

2. Materials Provided

BIONOTE FMD Type O Ab ELISA contains following items to perform the assay.

1	Antigen coated Microplate (1)	96 wells/plate, configured in twelve 1x8 strip
2	Negative Control (2)	Normal bovine serum and Proclin 300 (0.05%)
3	Positive Control (3)	Antibody to FMDV and Proclin 300 (0.05%)
4	Validation Control 1 (4)	Monoclonal anti-FMDV-HRP, phosphate buffered saline, BSA and Proclin 300 (0.05%)
5	Validation Control 2 (5)	Monoclonal anti-FMDV-HRP, phosphate buffered saline, BSA and Proclin 300 (0.05%)
6	10X Washing Solution (6)	PBS-Tween 20 and Proclin 300 (0.05%)
7	101X Enzyme Conjugate (7)	Monoclonal anti-FMDV-HRP, phosphate buffered saline, BSA and Proclin 300 (0.05%)
8	Conjugate Diluent (8)	Phosphate buffered saline and Proclin 300 (0.05%)
9	TMB Substrate (9)	Tetramethyl-benzidine with citrate-phosphate buffer containing hydro-peroxide (H_2O_2): STORE IN THE DARK. Ready to use.
10	Stop Solution (10)	1N sulfuric acid. Ready to use.
11	Adhesive Plate Sealer (11)	

3. Materials Needed, but Not Provided

1) Dilution microplate or disposable test tube
2) Micro pipette
3) ELISA Washer
4) ELISA Reader

4. Precautions

In order to obtain reproducible results, the following rules must be observed.

1) For *in vitro* diagnostic use only.
2) Store the components at 2~8 ℃ right after use. Do not reuse microwells or pour reagents back into their original bottles once dispensed.
3) Do not inter-mix components from kits with different batch numbers.
4) Do not use reagents after the expiry date.
5) Do not reuse containers and residues to avoid contamination of each reagent with sample or other reagents.
6) Handle all reagents and samples as bio-hazardous materials.
7) Use fresh samples. Hemolyzed or contaminated samples may give erroneous results.
8) Remove the blood corpuscle in samples clearly. It may give non-specific reaction.
9) Wear the gloves when you handle the potentially infectious materials. Wash hands thoroughly after the tests are done.
10) Keep all reagents away from skin and eyes. If exposure should occur, rinse immediately with fresh cold water.
11) Dispose of containers and residues safely in accordance with national and local regulations.
12) TMB Substrate (9) and Stop Solution (10) can cause irritation or burns to the skin and eyes. In case of an accident, rinse immediately with fresh cold water.
13) Please reset the test conditions before using an automated anaylzer as the test results may vary.

5. Specimen Collection and Storage

1) Either fresh serum or plasma samples from cattle, pigs, or goats can be used for this assay. Any visible particulate matters in the sample should be removed by centrifugation at 3,000 rpm for at least 20 minutes.
2) If samples are not immediately tested, they should be refrigerated at 2~8 ℃ or up to 2 weeks. For longer storage, freeze the samples at -20 ℃ or below. They must be at room temperature (18~25 ℃) for 30 minutes before beginning the test procedure.

[Preparing of plasma]

① Blood should be collected with a disposable syringe and added to a tube containing anticoagulant (Heparin, EDTA, or Citrate), and then separate plasma by centrifugation at 3,000 rpm for at least 20 minutes.
② Plasma may be stored at 2~8 ℃ up to 2 weeks, for longer storage (1 year) freeze at below -20 ℃ .

[Preparing of serum]

① Blood should be collected with a disposable syringe and added to a serum collection tube (no anticoagulant).
② Leave the collected blood at room temperature for 30 minutes to coagulate, and then separate serum by centrifugation at 3,000 rpm for at least 20 minutes.
③ Serum may be stored at 2~8 ℃ up to 2 weeks, for longer storage (1 year) freeze at below -20 ℃.

6. Preparation of Reagent and Samples

1) Allow all reagents and samples to equilibrate at room temperature (18~25 ℃) for 30 minutes before use.
2) Preparation of working Enzyme Conjugate: The 101X Enzyme Conjugate (7) must be diluted 1 to 100 with Conjugate Diluent (8) before use. (Dilute 10 ㎕ Enzyme Conjugate (7) in 1 ml of Conjugate Diluent (8) (1:100 dilution). Mix well.)
3) Preparation of diluted Washing Solution: The 10X Washing Solution (6) must be diluted 1 to 9 with distilled/deionized water before use. (e.g. Dilute 100 ml 10X Washing Solution (6) in 900 ml of distilled/deionized water (1:9 dilution) and mix well). Crystals in 10X Washing Solution (6) might be showed if stored at cold temperature. It's not a quality problem. Use the solution after dissolving crystals by placing the vial at 37 ℃ for few minutes.
4) Stability of Prepared Reagent

Reagent	State	Storage	Stability
Working Enzyme Conjugate	Once prepared	2~8 ℃	8 hours
Working Washing solution	Once prepared	2~30 ℃	1 week

7. Procedure of the Test

1) Allow all reagents and samples to equilibrate at room temperature (18~25 ℃) for 30 minutes and shake them gently before use.
2) Prepare the strip wells for Negative Control (2), Positive Control (3) and each of the samples.
3) Dispense **25 ㎕ of Negative Control (2)** into three wells.

4) Dispense **25 ㎕ of Positive Control (3)** into two wells.
5) Dispense **25 ㎕ of Validation Control 1 (4), Validation Control 2 (5) and samples** into appropriate wells.
6) Dispense **100 ㎕ of working Enzyme Conjugate** into corresponding wells.
7) Shake the Antigen coated Microplate (1) gently and cover the plate(s) with an Adhesive Plate Sealer (11). Shaking is very important to get the reproducible results.
8) Incubate the plate(s) for **90 minutes at 37±1 ℃.**
9) Aspirate the liquid contents of all wells. Wash the plate(s) 6 times with **350 ㎕ of diluted Washing Solution**. Tap the plate(s) firmly after the last washing.
10) Dispense **100 ㎕ of TMB Substrate (9)** into each well right after removing Washing Solution. (If over 5 minutes delayed, it might cause the OD value decrease.)
11) Incubate the wells for **15 minutes at room temperature (18~25 ℃)** IN THE DARK.
12) Dispense **100 ㎕ of Stop Solution (10)** into each well.
13) Blank the spectrophotometer on air.
14) Measure and record the absorbance of the samples and controls at 450 nm in a bichromatic spectrophotometer (with reference wavelength at 620 nm) right after the end of assay, within 10 minutes.
15) Calculate the results.

8. Interpretation of the Test

1) Test validation

① The mean absorbance value of Negative Controls (NCx) must be ≥ 1.0 and < 2.3.
② The mean absorbance value of Positive Controls (PCx) must be ≤ 0.2 and the PI value of positive control must be ≥ 80.
The PI value of Validation Control 1 (VC1) must be > 60.
The PI value of Validation Control 2 (VC2) must be < 40.
③ If these values are out of range, result should be considered invalid and the samples should be retested.
④ If the OD_{450} of a test sample is higher than the mean OD_{450} of negative control, the Percentage Inhibition can be interpreted as 0%.

2) Calculation of Results

Calculate the mean of Negative Controls absorbance. Then calculate the PI (Percent inhibition) value of Positive Controls and each test sample using the following formula of percent inhibition.

PI value = [1-(OD_{sample} / NCx)] x 100

3) Interpretation of Results

The status of samples is determined as follows;

- **Positive**: PI value ≥ 50%
  The sample is regarded as positive for FMDV Type O SP Ab.
- **Negative**: PI value < 50%
  The sample is regarded as negative for FMDV Type O SP Ab.

For example,
- PCx: 0.028, NCx: 2.013, VC1: 0.702, VC2: 1.305
- OD_{450} of sample: 0.562
- PI value of PCx = [1-(0.028/2.013)] x 100 = 98.61 (Validation: ≥ 80)
- PI value of VC1 = [1-(0.7021/2.013)] X 100 = 65.1 (Validation: > 60)
- PI value of VC2 = [1-(1.305/2.013)] X 100 = 35.2 (Validation: < 40)
- PI value of sample = [1-(0.562/2.013)] x 100 = 72.1 (Positive ≥ 50)

→ This sample is considered as positive

* As other diagnostic tests, a definitive diagnosis should be determined by a clinician after all clinical and laboratory findings have been evaluated.

9. Limitations and Interferences

1) The BIONOTE FMD Type O Ab ELISA can detect FMDV Type O SP Ab in cattle, pigs, and goats. It can't differentiate between vaccinated and infected animals.
2) Samples
① Pasteurized samples (no less than 10 hours at 60 ℃) may lead to diminished reactivity and therefore should not be used.
② Anticoagulants such as heparin, EDTA, and citrate do not affect the test result.
③ Hemolytic samples should be centrifuged before use to avoid interference by cellular constituents.
3) Failure to add specimen in the procedure could result in a falsely negative test result. Repeating the test should be considered where there is clinical suspicion of infection.

10. Storage and Stability

1) All reagents should be stored at 2~8 ℃. Do not freeze.
2) Shelf life is up to 12 months. This test kit is stable through the expiration date printed on the package and on the label of each material/reagent as unopened state.

11. Packaging Unit

Reagent \ Volume	96 Tests/Kit	480 Tests/Kit	960 Tests/Kit
Antigen coated Microplate (1)	1 plate	5 plates	10 plates
Negative Control (2)	0.3 ml/vial x 1	1 ml/vial x 1	2 ml/vial x 1
Positive Control (3)	0.3 ml/vial x 1	1 ml/vial x 1	2 ml/vial x 1
Validation Control 1 (4)	0.3 ml/vial x 1	1 ml/vial x 1	2 ml/vial x 1
Validation Control 2 (5)	0.3 ml/vial x 1	1 ml/vial x 1	2 ml/vial x 1
10X Washing Solution (6)	50 ml/bottle x 1	250 ml/bottle x 1	250 ml/bottle x 2
101X Enzyme conjugate (7)	0.3 ml/bottle x 1	1.2 ml/bottle x 1	2.5 ml/bottle x 1
Conjugate Diluent (8)	15 ml/bottle x 1	80 ml/bottle x 1	200 ml/bottle x 1
TMB Substrate (9)	12 ml/bottle x 1	60 ml/bottle x 1	120 ml/bottle x 1
Stop Solution (10)	15 ml/bottle x 1	80 ml/bottle x 1	150 ml/bottle x 1
Adhesive Plate Sealer (11)	2 ea	10 ea	20 ea
Instructions for Use (12)	1 ea	1 ea	1 ea

12. Precision

Within-run and between-run precisions have been determined by the testing 10 replicates of three specimens: a negative serum and a positive serum. The C.V (%) of negative and positive values were within 10% of the time.

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