

Foot and Mouth Disease Virus Antibody ELISA



BIONOTE FMD NSP Ab ELISA 2.0

Principle of the Test

The BIONOTE FMD NSP Ab ELISA 2.0 is a Competitive Enzyme Linked Immunosorbent Assay for the qualitative detection of antibody (Ab) against FMDV non-structural protein (NSP) in serum or plasma of cattle, pigs, and goats. It contains a microplate, which is pre-coated with recombinant NSP 3ABC antigen on the well. For testing, ELISA plates coated with the 3ABC are incubated with an equal mixture of sample and monoclonal antibody-HRP for 90 minutes at 37°C. During the incubation, if there are FMDV NSP Abs in the test sample, the antibodies and HRP conjugated monoclonal antibodies against FMDV NSP competitively bind to the antigens on the well. After incubation, all unbound materials are removed by washing step and substrate is dispensed. 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 NSP 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 3ABC antigens are used as capture material in this test. These enable the FMD NSP Ab ELISA 2.0 to identify FMDV NSP Abs in a sample with a high degree of accuracy.

Materials provided

Antigen coated Microplate (1)	96 wells/plate, configured in twelve 1x8 strip. Recombinant 3ABC antigen is coated.
Negative Control (2)	Normal bovine serum and Proclin 300 (0.05%)
Positive Control (3)	Rabbit polyclonal antibody to FMDV and Proclin 300 (0.05%)
10X Washing Solution (4)	PBS-Tween 20 and Proclin 300 (0.05%)
101X Enzyme Conjugate (5)	Monoclonal anti-FMDV-HRP, BSA and stabilizers Preservative is Proclin 300 (0.05%)
Conjugate Diluent (6)	Phosphate buffered saline, BSA and stabilizers Preservative is Proclin 300 (0.05%)
TMB Substrate (7)	Tetramethyl-benzidine with citrate-phosphate buffer containing hydro-peroxide (H ₂ O ₂): STORE IN THE DARK. Ready to use.
Stop Solution (8)	1N hydro chronic acid. Ready to use.

Materials required, but not provided

- Precision pipettes or multiple delivery pipetting devices suitable for delivering 10 to 1000 μ l
- Disposable pipette tips
- 500 ml graduated cylinder for washing solution
- 96 well plate reader
- Distilled or deionized water
- Vortex mixer

Precautions

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

- 1) Use fresh sample. Hemolyzed or contaminated sample might cause false result.
- 2) Remove the blood corpuscle in samples before use. They may cause non-specific reaction.
- 3) Use disposable gloves while handling potentially infectious material and performing the assay. After assay, wash hands with sanitizers.

- 4) Store all reagents at 2~8°C (35~46°F) in the dark. Bring to room temperature (18~25°C) prior to use, and return to 2~8°C (35~46°F) following use.
- 5) Unused microplate wells should be stored sealed in the enclosed plastic bag at 2~8°C. It should be used as soon as possible. Do not reuse microwells or pour reagents back into their original bottles once dispensed.
- 6) Do not intermix components from kits with different batch numbers.
- 7) TMB Substrate (7) and Stop Solution (8) can cause irritation or burns to the skin and eyes. In case of accident, rinse immediately with fresh cold water.
- 8) Do not expose TMB Substrate (7) to the light directly or any oxidizing agents. Handle all substrate solutions with new clean glass or plastic ware.
- 9) Do not use reagents after the expiry date.
- 10) Do not reuse containers and residues, so avoid contamination of each reagent with sample or other reagents.
- 11) Optimal results will be obtained by strict adherence to this protocol. Careful pipetting, timing and washing throughout this procedure are necessary to maintain precision and accuracy.
- 12) Dispose of containers and residues safely in accordance with national and local regulations.
- 13) Please reset the test conditions before using an automated analyzer, as the test results may vary.

Collection and Storage of Specimen

- 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°C for up to one week. For longer storage, freeze the samples at -20°C or below. They must be at room temperature (18~25°C) for 30 minutes before beginning the test procedure.

Preparation of Reagents

- 1) Allow all reagents to equilibrate at room temperature (18~25°C) for 30 minutes before use.
- 2) **Preparation of working Enzyme Conjugate:** The 101X Enzyme Conjugate (5) must be diluted 1 to 100 with Conjugate Diluent (6) before use. (Dilute 10 μ l 101X Enzyme Conjugate (5) in 1 ml of Conjugate Diluent (6) (1:100 dilution). Mix well.)
- 3) **Preparation of working Washing Solution:** The 10X Washing Solution (4) must be diluted 1 to 9 with distilled/deionized water before use. (e.g. Dilute 50 ml 10X Washing Solution (4) in 450 ml of distilled/deionized water (1:9 dilution) and mix well). Crystals in 10X Washing Solution (4) 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°C for few minutes.
- 4) **Stability of Prepared Reagent**

Reagents	State	Storage	Stability
working Enzyme Conjugate	Once prepared	2~8°C	8 hours
working Washing Solution	Once prepared	2~30°C	1 week

Procedure of the Test

- 1) Allow all reagents and samples to equilibrate at room temperature (18~25°C) 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 50 μ l of Negative Control (2)** into three wells.
- 4) **Dispense 50 μ l of Positive Control (3)** into two wells.
- 5) **Dispense 50 μ l of samples** into appropriate wells.
- 6) **Dispense 50 μ l of working Enzyme Conjugate** into corresponding wells.
- 7) Shake the plate(s) gently and cover the plate(s) with an Adhesive Plate Sealer. Shaking is very important to get the reproducible results.
- 8) Incubate the plate(s) for **90 minutes** at **37 \pm 1°C**.
- 9) Aspirate the liquid contents of all wells. Wash the plate(s) 6 times with **350 μ l of working Washing Solution**. Tap the plate(s) firmly after the last washing.
- 10) **Dispense 100 μ l of TMB Substrate (7)** 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°C) **IN THE DARK**.
- 12) **Dispense 100 µl of Stop Solution (8)** 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 30 minutes.
- 15) Calculate the results.

Interpretation of the Result

1) Test validation

- ① The mean OD₄₅₀ of the negative control (NCx) must be ≥ 1.0.
- ② The mean OD₄₅₀ of the positive control (PCx) must be ≥ -0.005 and ≤ 0.5000.
- ③ If either of these values is out of range, the test result should be considered as invalid and the samples should be retested.
 - ✓ If the mean OD₄₅₀ of a test sample is higher than negative control (NCx), the percentage inhibition (PI) can be interpreted as 0%.

2) Interpretation of the result

- ① Calculate the NCx obtained from duplicate wells. Then calculate the PI value of each test sample using the following formula.

$$\text{PI value} = [1 - (\text{OD}_{450} \text{ of sample} / \text{NCx})] \times 100$$

- ② Based on the PI value and species, the sample results are interpreted as follows:

Species	Cattle	Pig	Goat
Positive PI	Over 50	Over 50	Over 50
Negative PI	Below 50	Below 50	Below 50

- ③ For example

- NCx : 1.291, PCx : 0.211, OD₄₅₀ of sample : 1.250
- PI value of sample = $[1 - (1.250 / 1.291)] \times 100 = 3.2$
- The result of sample is interpreted as negative.

Limitations and interferences

- 1) For *in vitro* diagnostic use only.
- 2) Failure to add sample in the procedure could result in a falsely negative test. Repeat testing should be considered where there is clinical suspicion of infection.
- 3) Other clinically available tests are required if questionable results are obtained. As other diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test. It is recommended the diagnosis decision is made by the clinician after all clinical and laboratory findings have been evaluated.

Stability and Storage

- 1) The ELISA kit should be stored at 2~8°C. This test kit is stable through to the expiration date printed in the package and in the label of each material / reagent in an unopened state. The shelf life is 18 months.

Packaging unit

Reagent \ Volume	96 Tests/Kit	480 Tests/Kit	960 Tests/Kit
Antigen coated Microplate (1)	1 ea	5 ea	10 ea
Negative Control (2)	0.3 ml/vial x 1	1.5 ml/vial x 1	3.0 ml/vial x 1
Positive Control (3)	0.3 ml/vial x 1	1.5 ml/vial x 1	3.0 ml/vial x 1
10X Washing Solution (4)	50 ml/bottle x 1	250 ml/bottle x 1	250 ml/bottle x 2
101X Enzyme Conjugate (5)	8 ml/bottle x 1	40 ml/bottle x 1	80 ml/bottle x 1
Conjugate Diluent (6)	0.3 ml/vial x 1	1.2 ml/vial x 1	3.0 ml/vial x 1
TMB Substrate (7)	12 ml/bottle x 1	60 ml/bottle x 1	120 ml/bottle x 1

Stop Solution (8)	15 ml/bottle x 1	80 ml/bottle x 1	150 ml/bottle x 1
Adhesive Plate Sealer	2 ea	10 ea	20 ea
Instructions for Use	1 ea	1 ea	1 ea

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