

Classical Swine Fever Virus Antibody ELISA



BIONOTE CSFV Ab ELISA

■ Principle of the Test

The BIONOTE CSFV Ab ELISA is a blocking Enzyme Linked Immunosorbent Assay for the qualitative detection of specific antibodies to Classical Swine Fever Virus (CSFV) which is the most common and prevalent virus, in Swine serum.

The BIONOTE CSFV Ab ELISA contains a Microplate, which is pre-coated with recombinant E2(glycoprotein 55) on the well. For testing, ELISA plates are incubated with a serum and control (1:1 dilution with the Sample Diluent) for 60 minutes at 37 °C. During first incubation, anti-CSFV antibodies present in sample bind onto the antigen coated on the well. After incubation and washing step, then mAb-HRP is dispensed into the wells and incubated for 30 minutes at 37 °C. Following this incubation, all unbound materials are removed by washing step. The enzyme linked to the complex is revealed by addition of a TMB Substrate. The enzyme activity will thus be in inverse proportion to the anti-CSFV antibodies in samples and evidenced by incubating the solid-phase with a TMB Substrate for 15 minutes at room temperature (18~25 °C). The reaction is stopped by adding 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 E2(gp55) antigens are used as capture material in test. These enable the BIONOTE CSFV Ab ELISA to identify to anti-CSFV antibodies in pig serum, with a high degree of accuracy.

■ Materials Provided

BIONOTE CSFV Ab ELISA contains following items to perform the assay.

- 1) Antigen Coated Microplate (1)
- 2) Negative Control (2)
- 3) Positive Control (3)
- 4) Sample Diluent (4)
- 5) 20X Washing Solution (5)
- 6) Enzyme Conjugate (6)
- 7) TMB Substrate (7)
- 8) Stop Solution (8)
- 9) Adhesive Plate Sealer (9)
- 10) Instructions for use (10)

■ Precautions for Use

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 °C right after use. Do not reuse microwells or pour reagents back into their original bottles once dispensed.
- 3) Do not intermix components from kits with different batch numbers.
- 4) Do not use reagents after the expiry date.

- 5) Do not reuse containers and residues, so avoid contamination of each reagent with sample or other reagents.
- 6) Handle all reagents and samples as biohazardous 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. After handling, wash hands with sanitizers.
- 10) Keep all reagents away from skin and eyes. If exposure should occur, immediately rinse with fresh cold water.
- 11) Dispose of containers and residues safely in accordance with national and local regulations.
- 12) 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.
- 13) Strictly follow the test procedures to minimize false or invalid test results due to improper administration of the product usage or doses.
- 14) To prevent contamination, it is recommended to dispense the reagents required for testing in a clean, disposable container.
- 15) Take out as many plates as needed for use, place the remaining plates back in the zipper bag, then seal immediately, and store it in refrigerated conditions (2~8 °C). Use the microplates as soon as possible.
- 16) All samples and reagents must be placed at room temperature (18~25 °C) 30 minutes prior to use, and should only be used after confirming that they have acclimated to room temperature (18~25 °C).

■ Collection and Storage of Sample

- 1) Fresh pig serum samples should be used for this assay. Hemolyzed or contaminated samples may give erroneous results.
- 2) If samples are not immediately tested, they should be stored at 2~8 °C. For keeping samples more than 3 days, freeze the sample at -20 °C or below. They should be brought to room temperature before use.
- 3) Heat inactivated serum (for 30 min. at 56 °C) is available.
- 4) Sodium azide in sample may affect the test result.

■ Preparation of Reagent and Samples

- 1) Allow all reagents and samples to come to room temperature (18~25 °C) for 30 minutes before use.
- 2) Unused microplate wells must be sealed with silica gel in enclosed sealing bag and stored at 2~8 °C.
- 3) Mix samples thorough by gentle inversion. If necessary, any visible particulate matters in the samples should be removed by low-speed centrifugation.
- 4) **20X Washing Solution (5)** : Dilute the 20X Washing Solution (5) by distilled/deionized water (1:19). Add 50 ml of Washing Solution to 950 ml of distilled/deionized water and mix thoroughly. If undissolved crystals are present, re-suspend the solution by warming the bottle at 37 °C for 30 minutes. Store at 2~30 °C.

■ Procedure of the Test

- 1) Dispense 50 μl of the Sample Diluent (4) into each well of the Microplate (1). Run each control in duplicate.
- 2) Dispense 50 μl of the Positive Control (3), Negative Control (2) and test sample into well containing Sample Diluent (4). Mix well on microplate shaker.
- 3) Cover the wells with Plate Sealer (9) and incubate for 60 minutes at 37 °C.
- 4) Aspirate all liquid from the wells and rinse the wells five times with 350 μl of diluted Washing Solution. Remove any remaining Washing Solution by inverting the plate and blotting it against a clean paper towel.
- 5) Dispense 100 μl of Enzyme Conjugate (6) into each well.
- 6) Cover the wells with Plate Sealer (9) and incubate for 30 minutes at 37 °C.
- 7) Wash the wells as described above in 'Step 4'.
- 8) Dispense 100 μl of TMB Substrate (7) to each well.
- 9) Cover the wells with Plate Sealer (9) and incubate for 15 minutes at room temperature (18~25 °C) in the dark.
- 10) Add 100 μl of Stop Solution (8) to each well. Mix by gentle shaking.
- 11) Read the absorbance values of the wells at 450 nm in a bichromatic spectrophotometer (with reference wavelength at 620 nm) right after from the end of assay, within 30 minutes.

■ Interpretation of the Results

1) Test Validation

- ① The mean absorbance value of positive control (OD_{PCx}) is ≤ 0.2 .
- ② The mean absorbance value of negative control (OD_{NCx}) is ≥ 1.0 .
- ③ The PI value of positive control is ≥ 80 .
- ④ If these values are out of range, result should be considered invalid and the samples should be retested.
- ⑤ If the OD_{sample} is higher than the mean OD_{NCx} , the Percentage Inhibition can be interpreted as 0 %.

2) Calculation of the Result

Result is determined by PI value in the following manner.

$$\text{PI value} = \left[1 - \frac{OD_{\text{sample}}}{OD_{NCx}} \right] \times 100$$

For example,

- OD_{PCx} : 0.028, OD_{NCx} : 2.013, OD_{sample} : 0.562
- PI value of positive control = $[1 - (0.028/2.013)] \times 100 = 98.61$ (Validation: ≥ 80)
- PI value of sample = $[1 - (0.562/2.013)] \times 100 = 72.1$ (Positive ≥ 40)
- This sample is considered as positive

3) Interpretation of Results

The status of samples is determined as follows;

- PI value ≥ 40 is considered positive.
- PI value < 40 is considered negative.

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

* Sensitivity: 99.0 %, Specificity: 98.8 % (with 379 samples)

■ Stability and Storage

- 1) All reagents should be stored at 2~8 °C. Do not freeze.
- 2) Shelf life is 18 months. Use all reagents before the expiry date on the kit.
- 3) Antigen coated microplate can be used for up to 3 months after being removed from the zipper bag, as long as they are stored under refrigerated conditions (2~8 °C).
- 4) Stability of once prepared reagents

Reagent	State	Storage	Stability
Working Washing solution	Once prepared	2~30 °C	1 week

■ Packaging Unit

Reagent	Volume	96 Tests/Kit	480 Tests/Kit
Antigen Coated Microplate (1)		1 ea	5 ea
Negative Control (2)		0.5 ml/vial x 1	1.5 ml/vial x 1
Positive Control (3)		0.5 ml/vial x 1	1.5 ml/vial x 1
Sample diluent (4)		10 ml/bottle x 1	50 ml/bottle x 1
20X Washing Solution (5)		50 ml/bottle x 1	250 ml/bottle x 1
Enzyme Conjugate (6)		15 ml/bottle x 1	80 ml/bottle x 1
TMB Substrate (7)		12 ml/bottle x 1	60 ml/bottle x 1
Stop Solution (8)		15 ml/bottle x 1	80 ml/bottle x 1
Adhesive Plate Sealer (9)		2 ea	10 ea
Instruction for use (10)		1 ea	1 ea

Doc. No.: I4413-12E

Revised Date: Mar. 25, 2024



Manufactured by

BIONOTE, Inc.

22, Samsung 1-ro 4-gil, Hwaseong-si, Gyeonggi-do, 18449, Republic of Korea
TEL: 82-31-211-0516 | FAX: 82-31-8003-0618 | www.bionote.co.kr