Classical Swine Fever Virus Antibody ELISA

BIONOTE CSFV Ab ELISA

1. 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 diluents) for 60 minutes at 37℃. 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℃. Following this incubation, all unbounded materials are removed by washing step. The enzyme linked to the complex is revealed by addition of a substrate. The enzyme activity will thus be in inverse proportion to the anti-CSFV antibodies in specimens and evidenced by incubating the solid-phase with a substrate solution for 15 minutes at room temperature(18~25℃). The reaction is stopped by adding stop solution, and colorimetric reading will be performed by using a spectrophotometer at 450nm and reference wavelength at 620nm.

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.

2. Materials Provided

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

1	Antigen coated micro-assay plate	96 wells/plate, configured in twelve 1x8 strip Recombinant CSFV antigen(gp55) coated on the well.
2	Standard negative Control	SPF pig serum and proclin 300 (0.05%).
3	Standard positive Control	Anti-CSFV positive pig serum and proclin 300 (0.05%).
4	Sample diluents	Phosphate buffered saline and proclin 300(0.05%).
5	Washing solution (20X)	PBS-Tween 20 and proclin 300 (0.05%).
6	Enzyme conjugate	Anti-CSFV antibody - HRP and proclin 300 (0.05%). Ready to use.
7	TMB Substrate	Tetramethyl-benzidine with citrate-phosphate buffer containing hydro-peroxide (H_2O_2): STORE IN THE DARK. Ready to use.
8	Stopping solution	1N sulfuric acid. Ready to use.
9	Adhesive plate sealer	

3. 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℃ 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) Substrate and stopping solution can cause irritation or burns to the skin and eyes. In case of accident, rinse immediately with fresh cold water.

4. 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 refrigerated at 2~8℃. For longer storage, freeze the samples at -20℃ or below. Avoid repeated freezing and thawing.
3) Heat inactivated serum (for 30min at 56℃) is available.

5. Preparation of Reagent and Samples

1) Allow all reagents and samples to come to room temperature(18~25℃) before use.
2) Unused microplate wells must be sealed with silica gel in enclosed sealing bag and stored at 2~8℃.
3) Mix samples thorough by gentle inversion. If necessary, any visible particulate matters in the samples should be removed by low-speed centrifugation.
4) **Washing solution (20X concentrated):** Dilute the 20x washing solution by distilled/deionized water(1:19). Add 50 ㎖ of Washing solution to 950 ㎖ of distilled/deionized water and mix thoroughly. Store at 2-8℃ or room temperature(18~25℃) after use.

6. Procedure of the Test

1) Dispense 50ul of the sample diluents into each well of the plate. Run each control in duplicate.
2) Dispense 50ul of the positive control, negative control and test sample into well containing sample diluents. Mix well on microplate shaker.
3) Cover the wells with plate sealer and incubate for 60 minutes at 37±1℃.
4) Aspirate all liquid from the wells and rinse the wells five times with 350 ㎕ of diluted washing solution. Remove any remaining washing solution by inverting the plate and blotting it against a clean paper towel.
5) Dispense 100 ㎕ of enzyme conjugate(ready to use) into each well.
6) Cover the wells with plate sealer and incubate for 30 minutes at 37±1℃.

7) Wash the wells as described above in Step 4.
8) Dispense 100 ㎕ of substrate(ready to use) to each well.
9) Cover the wells with plate sealer and incubate for 15 minutes at room temperature(18 ~ 25℃) in the dark.
10) Add 100 ㎕ of stopping solution(ready to use) to each well. Mix by gentle shaking.
11) Read the absorbance values of the wells at 450nm in a bichromatic spectrophotometer(with reference wavelength at 620nm) right after from the end of assay, within 30 minutes.

7. Interpretation of the Results

1) Test Validation

① The mean absorbance value of positive control(PCx) is ≤ 0.2.
② The mean absorbance value of negative control(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_{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 the Result

Result is determined by PI value in the following manner.

$$\text{PI value} = \left[1 - \frac{OD_{450} \text{ of sample}}{\text{mean } OD_{450} \text{ of negative control}}\right] \times 100$$

For example,
- PCx : 0.028, NCx : 2.013, OD_{450} of sample : 0.562
- PI value of positive control = [1-(0.028/2.013)] x 100 = 98.61 (Validation: ≥ 80)
- PI value of sample = [1-(0.562/2.013)] x 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)

8. Stability and Storage

1) All reagents should be stored at 2~8℃. Do not freeze.
2) Shelf life is 18 months. Use all reagents before the expiry date on the kit.
3) Stability of once prepared reagents

Reagent	State	Storage	Stability
Working Washing solution	Once prepared	Room temp(18 ~ 25℃) or 2-8℃	1 week

9. Packaging Unit

Reagent \ Volume	96 Tests/Kit	480 Tests/Kit
Antigen coated micro-assay plate	1 ea	5 ea
Negative Control	0.5ml/vial x 1	1.5ml/vial x 1
Positive Control	0.5ml/vial x 1	1.5ml/vial x 1
Sample diluents	10ml/bottle x 1	50ml/bottle x 1
Washing solution (20X concentrated)	50 ml/bottle x 1	250ml/bottle x 1
Enzyme conjugate	15ml/bottle x 1	80ml/bottle x 1
Substrate	12ml/bottle x 1	60ml/bottle x 1
Stopping solution	15ml/bottle x 1	80ml/bottle x 1
Adhesive plate sealer	2 ea	10 ea

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Manufactured by BIONOTE
BioNote, Inc.
22 Samsung1ro 4-gil, Hwaseong-si, Gyeonggi-do 18449, Republic of Korea
TEL: 82-31-211-0516 I FAX : 82-31-8003-0618 I www.bionote.co.kr