Classical Swine Fever virus Antibody ELISA

AniGen CSFV Ab ELISA

1. Disease Information

Classical Swine Fever (CSF) is a viral disease which has recently caused very serious economic losses in European Union (EU). CSF virus infection occurs under natural conditions in all pigs, i.e. domestic pigs and wild boar, which are equally susceptible to CSF virus infection (BRUGH *et al*.,1964:DEPNER *et al*.,1995) and also causes poor welfare in affected populations. After implementation of effective control measures in farmed pig populations, several countries, including Australia, Canada, New Zealand, United States of America, succeeded in eradicating CSF. These measures succeeded in the absence of the disease in wild boar or feral pigs. In most other parts of the world the CSF virus is present, causing considerable economic damage. Within the EU, most Member States are practically free of the disease in domestic pigs. CSF is currently present in parts of the wild boar population in several region of EU Member States, i.e. in France, Germany and Italy. It is also present in countries of Central and Eastern Europe that are likely to join the EU as well as in other Central and Eastern European countries.

2. Explanation of the Test

The Anigen CSFV(*Classical Swine Fever Virus*) Ab ELISA is a Competitive Enzyme Linked Immunosorbent Assay for the qualitative detection of antibody to the most common and prevalent CSFV in Swine serum.

The Anigen CSFV E2 Ab ELISA contains a microplate, which is pre-coated with CSFV E2 antigen on the well. For testing, ELISA plates coated with the antigen(E2) are incubated with an equal mixture of serum and Mab-HRP(1:100 dilution in the conjugate diluent) for 90 minutes at 37℃. During first incubation, CSFV E2 antibodies present in test sample and HRP conjugated monoclonal antibodies competitively bind to the antigens in the well. Following this incubation, all unbounded material is removed by aspiration and washing before adding a substrate solution. The residual enzyme activity found in the well will thus be directly inverse proportion to the anti-CSFV E2 antibodies in specimens and evidenced by incubating the solid-phase with a substrate solution. The reaction is stopped by adding a stop solution and colorimetric reading will be performed by using a spectrophotometer at 450nm and 620nm.

The specially selected CSFV E2 antigens are used as capture material in test. These enable the Anigen CSFV E2 Ab ELISA to identify to *Classical Swine Fever Virus* antibodies in specimens, with a high degree of accuracy.

3. Materials Provided (480 Tests/Kit)

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

1) Antigen coated micro-assay plate: 96 wells/plate x 5 plates, configured in twelve 1x8 strips.
2) Negative Control : 1vial (1.5 ㎖) of normal swine serum. Procline 300 (0.05%) added as preservatives
3) Positive Control : 1vial (1.5 ㎖) of anti-CSFV positive swine serum. Procline 300 (0.05%) added as preservatives.
4) Washing solution (10X concentrated) : 1 bottle(250 ㎖) of PBS-Tween 20. Preservative is Proclin 300 (0.05%) Note: Before use, take one bottle content, and then fill-up to 2,500 ㎖ with distilled water. In presence of undissolved crystals, re-suspend the solution by placing the vial at 37℃ for few minutes.
5) Enzyme Conjugate (101X concentrated) : 1vial(1.2 ㎖) of anti-CSFV- HRP, BSA and stabilizers. Preservative is Proclin 300 (0.05%).
6) Conjugate Diluent : 1 bottle (40 ㎖) of phosphate buffered saline, BSA and stabilizers. Preservative : Proclin 300 (0.05%).
7) Substrate A : . 1 bottle (40 ㎖) of citrate-phosphate buffer containing H_2O_2
8) Substrate B : 1 bottle (40 ㎖) of tetramethyl-benzidine with citrate-phosphate buffer : STORE IN THE DARK
   Note : before use, make 1:1 mix with substrate A and substrate B.
9) Stopping solution : 1 bottle (80 ㎖) of 1N sulfuric acid. Ready for use.
10) Adhesive plate sealer.
11) Instructions for use.

4. Precautions

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

1) For in vitro diagnostic use only.
2) Do not mix reagent of different lots.
3) Use thoroughly cleaned glassware, free from contamination of metal ion or oxidating substances.
4) Use disposable gloves while handling potentially infectious material and performing the assay.
5) Substrate and stopping solution should be handled with care. Avoid contact with skin, eyes and mucous membranes. In case of accident, rinse thoroughly with running water.

5. Specimen Collection and Storage

1) Either fresh serum or plasma samples can be used for this assay. Blood collected by venipuncture should be allowed to clot naturally and completely. Any visible particulate matters in the sample should be removed by centrifugation at 3,000 rpm for at least 20 minutes.
2) If specimens are not immediately tested they should be refrigerated at 2~8℃. For storage periods greater than three days, freeze the specimen at -20℃ or below(serum, plasma). They should be brought to room temperature prior to use.
3) Specimens containing precipitate may yield inconsistent test results. Such specimens must be clarified prior to assaying.

6. Reagent preparation

1) Allow all reagents to come to room temperature before use.
2) Don't dilute the positive, negative and samples.
3) **Enzyme Conjugate (101X concentrated)**: The enzyme conjugate concentrate must be diluted 1 to 100 with conjugate diluent before use.
4) **Wash solution (10X concentrated)**: The wash concentrated must be diluted 1 to 20 with distilled/deionized water before use.
5) **Substrate solution** : The substrate A should be diluted 1 to 1 with substrate B before

7. Procedure of the Test

1) Prepare the strip wells for negative control 2 wells, positive control 2 wells and each of the samples to each wells
2) Add 50 ㎕ of each of the primary positive, negative control solution to 2 wells, and 50 ㎕ of each of the samples to each wells.
3) Add 50 ㎕ of anti CSFV-HRP(1:100 dilution in the conjugate diluent) each wells.
4) Cover the microplate with adhesive plate sealer and mix well on vibrating mixer. Mixing is very important to get the reproducible results.
5) Incubate the wells at 37±1℃ for 60 minutes.
6) Wash the wells at 6 times with 350 ㎕ of diluted washing solution. Aspirate all liquid from the wells.
7) Pipette 100 ㎕ of substrate to each well.
8) Incubate the wells for 15 minutes at room temperature(18 ~ 25℃).
9) Pipette 100 ㎕ of stopping solution to each well.
10) Read the absorbance of the wells with a bichromatic spectrophotometer at 450nm with reference wavelength at 620nm. Reading must be completed within 1 hour from the end of assay.

8. Interpretation of the Test

1) PI value calculation

① Calculate the mean absorbance of the negative controls, and then calculate the PI (Percent inhibition) value by each serum, using the formula percent inhibition

PI value = [1-(OD sample/mean OD negative)] x 100

For example,
- Mean OD negative : 1.892

- OD sample : 1.520
- PI value = [1-(1.520/1.892)] x 100 = 19.7 → This sample is considered as negative

② Interpretation of the PI value

Based on the PI value and the samples are classed as follows:

- Positive : PI ≥ 40%
- Negative : PI < 40%

3) Test Validation

① If the OD_{450} of a test sample is higher than the mean OD_{450} negative, the Percentage Inhibition can be interpreted as 0%.

② Valid CSFV Ab ELISA results are obtained when the average OD_{450} value of the negative control is more than 1.000 and the positive control value is less than 0.500. If either of these values are out of range, CSFV Ab ELISA should be considered invalid and the samples should be retested

9. Limitations and Interferences

1) The test procedure, precautions and interpretation of results sections for this test kit must be followed closely when testing.
2) Samples
   ① Samples containing sodium azide may affect the test result.
   ② Pasteurized samples (no less than 10 hours at 60℃) may lead to diminished reactivity and therefore should not be used.
   ③ Heat-inactivated samples (30 minutes at 56℃) do not impair the test.
   ④ Anticoagulants such as heparin, EDTA, and citrate do not affect the test result.
   ⑤ 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. Repeat testing should be considered where there is clinical suspicion of infection.

10. Storage

All reagents should be stored at 2~8℃.

11. Stability

1) Do not use after the stated expiry date.
2) Stability of once preparated reagents

Reagent	State	Storage	Stability
Working Conjugate	Once prepared	Room temp(18 ~ 25℃)	Within 1 hour
		2 ~ 8℃	4 hours
Working Washing solution	Once prepared	Room temp(18 ~ 25℃)	1 weeks
Working Substrate	Once prepared	Immediately use after making working substrate	

12. Packaging Unit

96 Tests/kit, 480 Tests/kit

13. Precision

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

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