HRPro® CSFV AB C-ELISA

CAT. NO. ES-CSF-02

GENERAL DESCRIPTION

Pestivirus, a genus of the family Flaviviridae, consists of the distinct pathogen of the farm animals, classical swine fever virus (CSFV), bovine viral diarrhea virus(BVDV), and border disease virus of sheep. CSFV cause a highly contagious and often fatal disease in pigs that is characterized by fever and hemorrhages and can run an acute or chronic case.

Pestiviruses are enveloped single stranded RNA virus that encodes a single polyprotein. Three envelope-associated glycoprotein (E protein), E0 , E1 and E2(gp55), in the order of their arrangement in the polyprotein. Antibodies that neutralize virus infectivity are directed against epitopes located on E2 glycoprotein.

The HRPro® CSFV AB C-ELISA is designed to detect presence of anti-E2 antibody in swine serum. It provides a rapid, simple and specific method for detecting antibodies against CSFV excluding cross reaction with antibody of other pestiviruses such as Bovine viral diarrhea virus (BVDV) and Border disease virus (BDV).

The HRPro® CSFV AB C-ELISA showed excellent correlation with Neutralizing peroxidase-linked assay (NPLA), an OIE prescribed method.

The HRPro® CSFV AB C-ELISA is performed in plate that is coated with recombinant gp55 purified by immunoaffinity technique. If anti-E2 glycoprotein antibodies present in the sample, color will not develop after addition of specific Anti-E2 monoclonal antibody conjugated with HRPO (horseradish peroxidase) and the TMB substrate.

The OD (optical density) of color development is inversely proportional to the amount of antibody specific to E2 present in the pig sera. The competition value (%) of antibody in the pig serum is obtained by comparing the OD that develops in wells containing the samples with the OD from the wells containing the positive and negative control sera.

KIT COMPONENTS

Reagents	192 tests	480 tests
① CSFV gp55 Coated Plate	2 plates	5 plates
② 10X Washing Buffer	120㎖ X 1	240㎖ X 1
③ Dilution Buffer	60㎖ X 1	60㎖ X 1
④ HRPO Anti-CSFV Conjugate (CSFV-CAB)	40㎖ X 1	70㎖ X 1
⑤ Positive Control, PC	1.0㎖ X 1	2.0㎖ X 1
⑥ Negative Control, NC	1.0㎖ X 1	2.0㎖ X 1
⑦ TMB Substrate	30㎖ X 1	70㎖ X 1
⑧ Stop Solution	20㎖ X 1	40㎖ X 1
⑨ Sealing Film	2 sheets	5 sheets
⑩ Introduction Manual	1 copy	1 copy

PREPARATION

1. All reagents must be allowed to come to room temperature (20~25℃) before use. Mix reagents by gentle swirling.
2. 1X washing buffer preparation
   1) Shake 10X Washing Buffer(②) gently.
   2) Dilute 1 part of 10X Washing Buffer(②) with 9 parts of deionized water. The diluted 1X washing buffer is stable for 2 weeks at room temperature (20~25℃).
3. Sample preparation
   1) Serum or plasma samples may be used in this assay.
   2) Use fresh samples for the best result. Serum samples can be stored at 2~8℃ for less than 3 days or –20℃ for a longer period. Do not freeze and thaw serum samples repeatedly. Sera with hemolysis or bacterial contamination are not suitable for the analysis!
   3) Visible solid materials in serum samples should be separated by centrifugation.
4. TMB Substrate (⑦) should be warmed up for 30 minutes at room temperature (20~25℃) before use (10㎖/plate). If stored at low temperature, the color development may be poor.

TEST PROCEDURE

1. Remove the Antigen Coated Plate (①) from protective foil pouch.
2. Dispense 50☒ of Dilution buffer(③) into each well of the CSFV gp55 Coated Plate.
3. Add 50☒ of samples, PC(⑤) and NC(⑥) appropriate wells of plate containing dilution buffer. Final dilution factor is 1:2. Use care not to spill samples from well to well.
4. Seal the plate and Incubate for 60 minutes or overnight at room temperature(25± 1.0℃).
   • Overnight reaction is recommended for more sensitive and accuracy test.
5. Wash each well 3 times with 1X washing buffer (300☒ per well). And remove contents of well at each washing steps. After final washing, hit plate to the paper towel briefly to remove solution.
6. Dispense 100☒ of HRPO Anti-CSFV conjugate (CSFV-CAB)(④) to each well.
7. Seal the plate and incubate for 30 minutes at room temperature(25± 1.0℃).
8. Wash each well as step 5.
9. Dispense 100☒ of TMB substrate(⑦ solution to each well.
10. Seal the plate and incubate for 15 minutes at room temperature. Check the density of color development by naked eyes.
11. Add 50☒ of Stop Solution (⑧) to each well of the plate. Shake the test plate shortly (5~10 sec.). Be careful not to spill.
12. Measure and record the A (450nm) for samples and controls immediately.
13. Validate and calculate the results.

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Plate template example (1-well Test)

	1	2	3	4	5	6	7	8	9	10	11	12
A	PC	PC	13	21	29	37	45	53	61	69	77	85
B	NC	NC	14	22	30	38	46	54	62	70	78	86
C	1	7	15	23	31	39	47	55	63	71	79	87
D	2	8	16	24	32	40	48	56	64	72	80	88
E	3	9	17	25	33	41	49	57	65	73	81	89
F	4	10	18	26	34	42	50	58	66	74	82	90
G	5	11	19	27	35	43	51	59	67	75	83	91
H	6	12	20	28	36	44	52	60	68	76	84	92

RESULT INTERPRETATION

1. Validate if the mean OD of the NC is higher than 0.50 and the mean OD of the PC is lower than 0.20. If these criteria are not met, the test are invalid and the samples must be retested.
2. Calculate the % competition (%PC) of sample by following the formula

$$\%PC = \frac{\text{NC mean OD} - \text{Sample OD}}{\text{NC mean OD} - \text{PC mean OD}} \times 100$$

3. Result interpretation
   1) Test samples having ≥40% %PC are positive.
   2) Test samples having < 40% %PC are negative.

%PC	Interpretation
%PC ≥ 40%	Positive
%PC < 40%	Negative

4. Example of result calculation and interpretation
   1) ODs of NC : 1.121 , 1.201
      Mean OD = (1.121 + 1.201) / 2 = 1.161 (valid)
   2) ODs of PC : 0.085, 0.091
      Mean OD = (0.085 + 0.091) / 2 = 0.088 (valid)
   3) OD of Sample : 0.431
      %PC of the sample = (1.161-0.431) / (1.161-0.088) X100 = 68.03%
   4) Result interpretation: Positive

PRECAUTIONS

1. All reagents must be allowed to come to room temperature (20~25℃) before use. Mix reagents by gentle swirling. After use, return to 2~8℃.
2. Read this instruction manual thoroughly and follow all steps strictly for successfuluse of the product.
3. All test samples should be considered potentially infectious and all items contacting the samples should be considered contaminated.
4. Do not use expired or contaminated reagents.
5. Do not use reagents from other kits or lots.
6. Do not mix reagents from different lots of this same product.
7. Do not expose the reagents to excessive heat or direct light during storage and incubation.
8. Incomplete washing adversely may affect the result and precision of the assay.
9. Avoid microbial contamination of the reagents.
10. Avoid contamination of the TMB Substrate(⑦) with the HRPO Anti-CSFV Conjugate(CSFV-CAB)(④).
11. Wear personal protective equipment (PPE) such as lab coat, goggle, and disposable gloves while performing the assay. Wash hands thoroughly afterwards.
12. Do not eat, drink, smoke or apply cosmetics where kit reagents are handled. Do not pipette by mouth.
13. Pipette tips must be changed after each pipetting step. Use a clean disposable pipette tip for all steps.
14. Use care not to spill samples from well to well.
15. Deionized water or equal must be used to prepare the washing buffer.
16. Unused strips should be stored in the sealed foil pouch at 2~8℃. Re-sealed strips are recommended to use within one week.
17. For veterinary use only.

STORAGE AND STABILITY

Store all reagents at 2~8℃. Do not freeze. Reagents remain stable until the expiration date when stored as instructed.

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QUICK PROTOCOL

CSFV gp55 Coated Plate

↓

Dispense 50 μℓ of
Dilution Buffer

↓

Add 50μℓ of Samples & PC, NC
(Dilution factor 1:2)

RT, 1hr

↓

Washing
3 times

Dispense 100 μℓ of
HRPO Anti-CSFV Conjugate

RT, 30min

↓

Washing
3 times

Dispense 100 μℓ of
TMB Substrate

RT, 15min

↓

Dispense 50μℓ of
Stop Solution

↓

Measure OD at 450nm