

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 diarrhoea 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 polypeptide. Three envelope-associated glycoprotein (E protein), E0, E1 and E2(gp55), in the order of their arrangement in the polypeptide. Antibodies that neutralize virus infectivity are directed against epitopes located on E2 glycoprotein.

HRPro® CSFV Antigen ELISA kit is designed to detect presence of antigens in samples from CSFV infected cells, leukocytes and tissue extracts of pig. It provides a rapid, simple and specific method for detecting CSFV antigens excluding cross reaction with other pestiviruses such as Bovine viral diarrhoea virus (BVDV) and Border disease virus (BDV).

HRPro® CSFV Antigen ELISA kit showed excellent correlation with Reverse Transcriptase Polymerase chain reaction (RT-PCR) and virus isolation.

But this kit does not available discriminate between vaccine strain from field strain.

The CSFV Antigen ELISA Kit is performed on the ELISA plate coated with anti-E2 monoclonal antibodies. Extracted and diluted sample from leukocytes, tissue homogenates or tissue cultures is added in the each well of plates.

If viral antigens (gp55) are present in a sample, color will develop after addition of peroxidase conjugated anti-E2 monoclonal antibody and TMB substrate. The interpretation of the result is obtained by comparing the optical density (OD), which develops in wells containing the samples with the OD from the wells containing the positive control.

KIT COMPONENTS

Reagents	192 tests
① Anti-CSFV E2 Ab Coated Plate	2 plates
② 10X Sample Treatment Buffer (STB)	30ml X 1
③ 10X Washing Buffer	120ml X 1
④ Dilution Buffer	60ml X1
⑤ 20X Whole Blood Treatment Buffer (WTB)	120mlX1
⑥ HRPO Anti-CSFV Conjugate (CSFV-AG)	40ml X 1
⑦ Strong Positive Control, SPC	2.0ml X 1
⑧ Weak Positive Control, WPC	2.0ml X 1
⑨ Negative Control, NC	2.0ml X 1
⑩ TMB Substrate	30ml X 1
⑪ Stop Solution	20ml X 1
⑫ Introduction Manual	1 Copy
⑬ Sealing Film	2 sheets

REAGENTS PREPARATION

- All reagents must be allowed to come to room temperature (20~25°C) before use. Mix reagents by gentle swirling.
- 1X Sample Treatment Buffer (1X STB) Preparation
 - Shake 10X Sample Treatment Buffer (STB) (②) gently.
 - Dilute 1 part of 10X Sample Treatment Buffer (1X STB) (②) with 9 parts of deionized water.
- 1X Whole Blood Treatment Buffer (WTB) Preparation
 - Shake 20X Whole Blood Treatment Buffer (WTB) (⑤) gently.
 - Dilute 1 part of 20X Whole Blood Treatment Buffer (WTB) (⑤) with 19 part of deionized water.
- 1X washing buffer preparation
 - Shake 10X Washing Buffer (③) gently.
 - Dilute 1 part of 10X Washing Buffer (③) with 9 parts of deionized water. The diluted 1X washing buffer is stable for 2 weeks at room temperature (20~25°C).
- TMB Substrate (⑩) should be warmed up for 30 minutes at room temperature (20~25°C) before use (10ml/plate). If stored at low temperature, the color development may be poor.

SAMPLE PREPARATION

Tissue, leukocyte, whole blood or cell culture samples can be tested. Use preferably fresh tissues but, if necessary, tissues can be stored at -70°C.

1. Tissue extracts:

Process one or two tissues from each animal submitted, preferably spleen, tonsil, lung, brain, mesenteric lymph node or kidney. Generally the most sensitive result could be obtained in samples from spleen and tonsil.

- 1) With scissors, cut 1-2 g of tissue into small pieces.
- 2) Place the tissue pieces into a mortar and add 3-5 ml 1X Sample Treatment Buffer (STB) with sea sand.
- 3) Grind tissues and incubate for 1 hour at room temperature with gentle agitation.
- 4) Centrifuge at 12,000Xg for 1 minute (or 1,500Xg for 10 min.) and use supernatant for test.

2. Leukocyte Concentrate

2.1. Leukocyte separation by Ficoll gradient centrifugation

- 1) Overlay carefully 3ml of heparin or EDTA anti-coagulated blood on the 3ml of Ficoll gradient media (ex, Histopaque-1077®) in a centrifuge tube.
- 2) Centrifuge at 1,000Xg for 10 minutes and collect about 1ml of leukocyte layer from centrifuge tube with pipette and mix with 0.5ml of PBS.
- 3) Centrifuge the leukocyte at 12,000Xg for 1 minute and discard the supernatant.
- 4) Re-suspend the pellet in 300µl of 1X STB and mix well on vortex and incubate for 1 hour at room temperature with gentle agitation.
- 5) Centrifuge the samples at 12,000Xg for 1 minute (or 1,500Xg for 10 min.) and use supernatant for test.

2.2. Leukocyte separation by LeucoSep®

- 1) Add 3ml of Ficoll gradient media (Histopaque-1077®) into LuecoSep® (Greiner) and centrifuge at 1,000Xg for 30 seconds.
- 2) Add 3ml of heparin or EDTA anti-coagulated blood into Luecosep® and centrifuge at 1,000Xg for 10minutes.
- 3) Phases are separate such as below example. Plasma – Leukocyte – Histopaque – Disk – Histopaque – RBC
- 4) Collect 1ml of leukocyte layer using a pipette and mix with 0.5ml of PBS.
- 5) Centrifuge the leukocyte at 12,000Xg for 1 minute and discard the supernatant.
- 6) Re-suspend the pellet in 300µl of 1X STB. Mix well on vortex and incubate for 1 hour at room temperature with gentle agitation.
- 7) Centrifuge the samples at 12,000Xg for 1 minute (or 1,500Xg for 10 min.) and use supernatant for test.

3. Whole blood:

Whole blood may be effective samples for screen examination but less sensitive than leukocyte concentrates. If there is positive reaction in whole blood samples, it should be confirmed by sample from leukocyte concentrate or tissue extract.

- 1) Mix whole blood gently by converting tube. If blood clot is found, it should be recollect the samples
- 2) Mix whole blood with 1:4 volume of whole blood treatment buffer (WTB) and incubate 10 minutes at room temperature.
ex) In conical tube: 1 ml whole blood and 4 ml WTB.
- 3) Centrifuge at 12,000Xg for 3-5minute (or 1,500Xg, 10min) and discard the supernatant.
- 4) Repeat process 1)~3) until the complete removal of red blood cell(RBC) in whole blood
- 5) Disperse the pellet and add equal volume of 1X STB (500µl in microtube). Mix well on vortex and incubate at room temperature for one hour with gentle agitation.
- 6) Centrifuge at 12,000Xg for 1 minute (or 1,500Xg for 10 min.) and use supernatant for test.

4. Cell Cultures

- 1) Remove the cell culture medium and collect the cells from the flask by cell scraper.
- 2) Centrifuge the cells for 10 minutes at 1,500Xg and discard the supernatant.
- 3) Add 300µl of 1X STB and mix by vortex and incubate for 1 hour at room temperature with gentle agitation.
- 4) Centrifuge at 12,000Xg for 1 minute (or 1,500Xg for 10 min.) and use supernatant for test.

TEST PROCEDURE

1. Remove the Anti-CSFV E2 Ab Coated Plate(①) from protective foil pouch.
2. Dispense 50µl of Dilution buffer(④) into each well of the Anti-CSFV E2 Ab Coated Plate.
3. Add 50µl of samples, SPQ(⑦), WPC(⑧) and NC(⑨) into 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 90 minutes at 37°C or overnight at room temperature (25±1.0°C)
5. Wash each well 3~5 times with 1X washing buffer (300µl 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 100µl of HRPO Anti-CSFV Conjugate (CSFV AG) (⑥) to each well.
7. Seal the plate and incubate for 60 min at 37°C.
8. Wash each well as step 5.
9. Dispense 100µl of TMB substrate (⑩) to each well.
10. Seal the plate and incubate for 10 minutes at room temperature (25±1.0°C). Check the density of color development by naked eyes.

11. Add 50☐ of Stop Solution (⑪) to each well of the plate. Shake the test plate by gently tapping shortly (5~10 sec). **Be careful not to spill.**
12. Measure and record the plate at 450nm wavelength immediately.
13. Validate and calculate the results.

Plate template example (1-well Test)

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

RESULT INTERPRETATION

1. Validate if the mean OD of the SPC is higher than 0.80 and the mean OD of the WPC is higher than 0.35. And the mean OD of the NC is lower than 0.30.
If these criteria are not met, the test are invalid and the samples must be retested.
2. Calculate the % Sample to positive ratio (%SP) of samples by following the formula

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

3. Result interpretation
 - 1) Test samples having $\geq 20\%$ %SP are positive.
 - 2) Test samples having 15~20% %SP are Suspected.
 - 3) Test samples having $< 15\%$ %SP are negative.

%SP	Interpretation
%SP $\geq 20\%$	Positive
%SP 15~20%	Suspected
%SP $< 15\%$	Negative

4. Example of result calculation and interpretation
 - 1) ODs of NC : 0.085 , 0.091
Mean OD = $(0.085 + 0.091) / 2 = 0.088$ (valid)
 - 2) ODs of SPC : 1.121, 1.201
Mean OD = $(1.121 + 1.201) / 2 = 1.161$ (valid)
 - 3) OD of Sample : 0.431
%SP of the sample = $(0.431 - 0.088) / (1.161 - 0.088) \times 100 = 31.97\%$
 - 4) Result interpretation: Positive

PRECAUTIONS

1. All reagents must be allowed to come to room temperature (20~25°C) before use. Mix reagents by gentle swirling. After use, return to 2~8°C.
2. Read this instruction manual thoroughly and follow all steps strictly for successful use 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(⑩) with the HRPO Anti-CSFV Conjugate (CSFV-AG) (⑥).
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°C. Re-sealed strips are recommended to use within one week.
17. For veterinary use only.

STORAGE AND STABILITY

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

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QUICK PROTOCOL

Anti - CSFV E2 Ab Coated Plate

Dispense 50 μl of
Dilution Buffer

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



37°C, 90min

Washing
3~5 times

Dispense 100 μl of
HRPO Anti - CSFV Conjugate



37°C, 60min

Washing
3~5 times

Dispense 100 μl of
TMB Substrate



RT, 10min

Dispense 50 μl of
Stop Solution

Reading
OD 450nm