HRPro® PRRSV AB ELISA

CAT. NO. ES-PRR-03

GENERAL DESCRIPTION

HRPro® PRRSV AB ELISA is a diagnostic kit for detection of antibodies generated by vaccination or infection of porcine reproductive and respiratory syndrome virus (PRRSV) in swine serum and plasma samples. The kit is an indirect ELISA that is specifically identified with PRRSV antibody bound recombinant PRRSV antigen using anti-swine labelled horseradish peroxidase. The test is possible for detection of antibodies of both PRRSV type I (European) and type II (American) and is suitable for herd profile.

KIT COMPONENTS

Reagents	480 tests
① PRRSV Antigen Coated Plate	5 plates
② 10X Washing Buffer	240mℓX1
③ Dilution Buffer	240mℓX1
④ HRPO Anti-Swine IgG Conjugate	70mℓX1
⑤ Positive Control, PC	7.0mℓX1
⑥ Negative Control, NC	7.0mℓX1
⑦ TMB Substrate	70mℓX1
⑧ Stop Solution	40mℓX1
⑨ User manual	1 copy

PREPARATION

1. Warm up all reagents at room temperature (20~25℃) for 30 minutes.
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. Serum dilution
   1) Use fresh serum 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!
   2) Visible solid materials in serum samples should be separated by centrifugation.
   3) Prepare 1㎖ deep-well-plate (DWP, 96-well, not offered) or suitable tubes.
   4) Dilute 5㎕ of test serum with 495㎕ of Dilution Buffer (③) in a DWP well or a suitable tube.
4. Do not dilute the Positive Control (PC, ⑤) and the Negative Control (NC, ⑥).
5. 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. Add 100㎕ of the diluted serum sample to each well of the plate, keeping the wells A1 and A2 for Positive Control (PC, ⑤) and the wells B1 and B2 for Negative Controls (NC, ⑥). Use care not to spill samples from well to well.
3. Add 100㎕ of undiluted Positive (PC, ⑤) and Negative (NC, ⑥) Controls in the designated wells.
4. Incubate the plate for 30 minutes (±2 min) at 25 ± 3 ℃.
5. Wash the plate three times with 300㎕ of 1X washing buffer. Get rid of moisture by tapping the plate on a dry paper towel.
6. Add 100㎕ of HRPO Anti-Swine IgG Conjugate (ready for use, ④) to each well.
7. Incubate the plate for 30 minutes (±2 min) at 25 ±3 ℃.
8. Wash the plate as described in Step 5.
9. Add 100㎕ of TMB Substrate (⑦) to each well.
10. Cover the plate and Incubate for 15 minutes (±1 min) at 25±3 ℃. Protect the plate from direct light exposure.
11. Add 50㎕ of Stop Solution (⑧) to each well of the plate. Shake the test plate shortly (5~10 sec.) either on an orbital shaker(~300rpm) or manually on the working bench. Be careful not to spill.
12. Read 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	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 PC (PCx) is higher than 0.40 and the mean OD of the NC (NCx) is lower than 0.30. If these criteria are not met, the test are invalid and the samples must be retested.
2. Calculate a S/P ratio as below.

$$S/P = \frac{\text{Sample OD} - \text{NCx}}{\text{PCx} - \text{NCx}}$$

3. Result interpretation
   1) Test samples having ≥ 0.4 S/P are positive.
   2) Test samples having < 0.4 S/P are negative.

S/P value	Interpretation
S/P ≥ 0.4	Positive
S/P < 0.4	Negative

4. Example of result calculation and interpretation
   1) ODs of PC : 1.121, 1.201
      Mean OD = (1.121 + 1.201) / 2 = 1.161 (valid)
   2) ODs of NC : 0.085, 0.091
      Mean OD = (0.085 + 0.091) / 2 = 0.088 (valid)
   3) OD of Sample : 0.870
      S/P ratio of the sample
      = (0.870 – 0.088) / (1.161 – 0.088) = 0.729
   4) Result interpretation: Positive

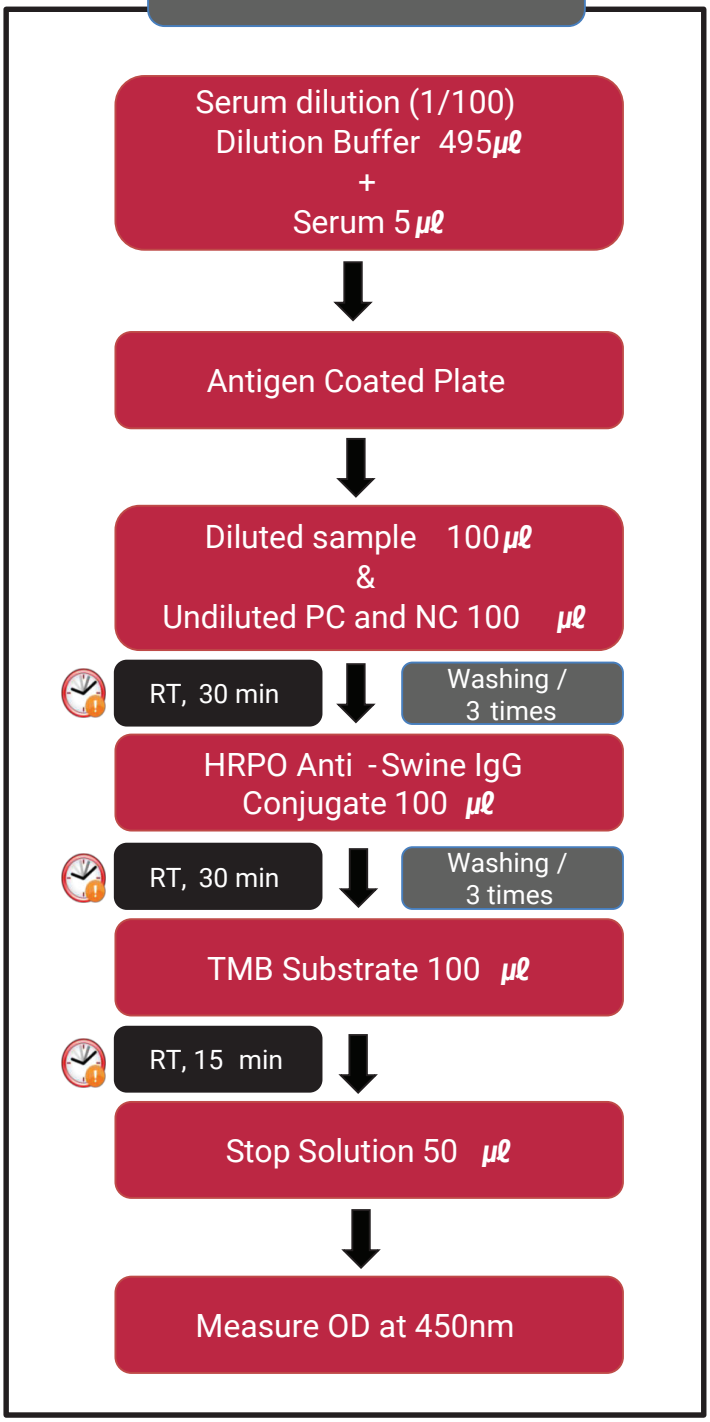
PRECAUTIONS

1. Bring the reagents to room temperature (20~25℃) for 30 minutes. 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-Swine IgG Conjugate(④).
11. Wear personal protective equipment (PPE) such as lab coat, goggle, and disposable gloves while performing the assay. Wash hands thoroughly afterwards.
12. Avoid microbial contamination of the reagents.
13. Avoid contamination of the TMB Substrate(⑦) with the HRPO Anti-Swine IgG Conjugate(④).
14. Do not eat, drink, smoke or apply cosmetics where kit reagents are handled. Do not pipette by mouth.
15. Pipette tips must be changed after each pipetting step. Use a clean disposable pipette tip for all steps.
16. Use care not to spill samples from well to well.
17. Deionized water or equal must be used to prepare the washing buffer.
18. Unused strips should be stored in the sealed foil pouch at 2~8℃. Re-sealed strips are recommended to use within one week.
19. 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.

QUICK PROTOCOL



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