HRPro® ADV AB Screen ELISA

CAT. NO. ES - ADV - 01

GENERAL DESCRIPTION

Aujeszky's disease (pseudorabies) is a highly contagious, economically significant disease of pigs. This viral infection tends to cause central nervous system(CNS) signs in young animals, respiratory illness in older pigs, and reproductive losses in sows. Aujeszky's disease results from infection by suid herpesvirus 1 (SuHV-1), which is also known informally as Aujeszky's disease virus(ADV).

The HRPro® ADV AB Screen ELISA is designed to detect presence of anti-ADV antibody in swine serum. It provides a rapid, simple and specific method for differentiating the antibodies against ADV infection or vaccination.

The HRPro® ADV AB Screen ELISA shows excellent correlation with virus neutralization (VN) test, a prescribed method by OIE, and has enough sensitivity to international standard reference serum.

The HRPro® ADV AB Screen ELISA is performed in plate that is coated with highly purified viral protein. If anti-ADV antibodies present in the sample, color will not develop after addition of specific anti-ADV monoclonal antibody conjugated with HRP (horseradish peroxidase) and the chromogen-containing substrate. The OD (optical density) of color development is inversely proportional to the amount of antibody specific to ADV present in the pig sera.

KIT COMPONENTS

Reagents	192 tests	480 tests
① ADV Antigen Coated Plate	2 plates	5 plates
② 10X Washing Buffer	120㎖ X 1	240㎖ X 1
③ Dilution Buffer	60㎖ X 1	100㎖ X 1
④ HRPO Anti-ADV Conjugate	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. Samples can be stored at 2~8℃ for less than 3 days or –20℃ for a longer period. Do not freeze and thaw 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) Dilute test samples two-fold (1:2) with dilution buffer (③). (e.g., by diluting 100㎕ of sample with 100㎕ of dilution buffer(③)). Diluted Samples should be mixed prior to dispensing into the Antigen Coated Plate(①).
4. Dilute PC (⑤) and NC (⑥) two-fold (1:2) with dilution buffer (③). Diluted PC and NC should be mixed prior to dispensing into the Antigen Coated Plate(①).
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. Dispense 100㎕ of diluted samples into appropriate wells. Use care not to spill samples from well to well.
3. Dispense 100㎕ of diluted NC (Diluted 1:2) into duplicate wells.
4. Dispense 100㎕ of diluted PC (Diluted 1:2) into duplicate wells.
5. Seal the plate and incubate for 60 minutes at room temperature (25± 1.0℃).
6. 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.
7. Dispense 100㎕ of HRPO Anti-ADV Conjugate (④) to each well.
8. Seal the plate and incubate for 20 minutes at room temperature (25± 1.0℃).
9. Wash each well as step 6.
10. Dispense 100㎕ of TMB substrate (⑦) to each well.
11. Seal the plate and incubate for 15 minutes at room temperature (25± 1.0℃). Check the density of color development by naked eyes.
12. Add 50㎕ of Stop Solution (⑧) to each well of the plate. Shake the test plate by gently tapping shortly (5~10 sec). Be careful not to spill.
13. Measure and record the A (450nm) for samples and controls immediately.
14. 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 0.70 and the mean OD of the PC is lower than 0.25. If these criteria are not met, the test are invalid and the samples must be retested.
2. Calculate the sample to negative ratio (SN) by following the formula

$$SN = \frac{\text{Sample OD}}{\text{NC mean OD}}$$

3. Result interpretation
   1) Test samples having ≤0.60 S/N are positive.
   2) Test samples having > 0.60 S/N are negative.

S/N value	Interpretation
S/N ≤ 0.60	Positive
S/N > 0.60	Negative

4. Example of result calculation and interpretation
   1) ODs of PC : 0.085, 0.091
      Mean OD = (0.085 + 0.091) / 2 = 0.088 (valid)
   2) ODs of NC :1.121, 1.201
      Mean OD = (1.121 + 1.201) / 2 = 1.161 (valid)
   3) OD of Sample : 0.431
      S/N value of the sample = (0.431 / 1.161) = 0.371
   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-ADV 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. 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

Samples, PC, NC Dilution (Dilution factor 1:2)
Dilution buffer 100 ㎕ + Samples, PC, NC 100 ㎕

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ADV Antigen Coated Plate

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Diluted Samples 100 ㎕
&
Diluted PC, diluted NC 100㎕

RT, 1hr → Washing 3 times

HRPO Anti - ADV Conjugate 100 ㎕

RT, 20min → Washing 3 times

TMB Substrate 100 ㎕

RT, 15min

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Stop Solution 50 ㎕

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Measure OD at 450nm