# VDPro® APP2 AB ELISA

**CAT. NO. ES-AP2-01**

## GENERAL DESCRIPTION

*Actinobacillus pleuropneumoniae* was found to be the causative agent for up to 20% of all bacterial pneumonia cases in swine.

VDPro® APP2 AB ELISA is an indirect enzyme-linked immunosorbent assay for the detection of antibodies against *Actinobacillus pleuropneumoniae* serotype 2 in swine serum samples.

VDPro® APP2 AB ELISA is based on OMP(membrane protein) antigen from *Actinobacillus pleuropneumoniae* serotype 2 and an effective tool to screen for infections and immunity levels through monitoring average antibody levels after *Actinobacillus pleuropneumoniae* serotype 2 (APP2) vaccination in swine herds.

## KIT COMPONENTS

| Reagents | 480 tests |
|---|---|
| ① APP2 Ag coated plate | 5 plates |
| ② 10X Washing Buffer | 240㎖ X 1 |
| ③ Dilution Buffer | 240㎖ X 1 |
| ④ HRPO Anti-Swine IgG Conjugate | 70㎖ X 1 |
| ⑤ Positive Control, PC | 7.0㎖ X 1 |
| ⑥ Negative Control, NC | 7.0㎖ X 1 |
| ⑦ TMB Substrate | 70㎖ X 1 |
| ⑧ Stop Solution | 40㎖ X 1 |
| ⑨ Sealing Film | 5 sheets |
| ⑩ Instruction Manual | 1 copy |

## PREPARATION

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. 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) Use the serum samples as fresh as possible
   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. Sample dilution
   1) Prepare the 1 ㎖ deep-well-plate (DWP, 96-well, not offered) or suitable tubes.
   2) Dilute the sample 1/100 in dilution buffer(③). Add 495 ㎕ dilution buffer(③) to each well of DWP and add 5 ㎕ serum to each well. Diluted Samples should be mixed prior to dispensing into the Antigen Coated Plate(①).
5. **Do not dilute the positive control(PC, ⑤) and negative control(NC, ⑥).**
6. 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 sample into appropriate wells. **Use care not to spill samples from well to well.**
3. Dispense 100㎕ of undiluted NC( ⑥ ) and undiluted PC(⑤) into duplicate wells.
4. Seal the plate and Incubate for 30 minutes at 25 ±1.0 ℃.
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-Swine IgG Conjugate (④) to each well.
7. Seal the plate and incubate for 30 minutes at 25 ±1.0 ℃.
8. Wash each well as step 5.
9. Dispense 100㎕ of TMB substrate (⑦) to each well.
10. Seal the plate and incubate for 15 minutes at 25±1.0℃. 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.

**Plate template example (1-well Test)**

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

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## RESULT INTERPRETATION

1. Validate if the mean OD of the PC is higher than 0.50 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 (S/P ratio) by following the formula

$$\text{S/P ratio} = \frac{\text{Sample OD} - \text{NC mean OD}}{\text{PC mean OD} - \text{NC mean OD}}$$

3. Result interpretation
   1) Test samples having ≥0.4 S/P ratio are positive.
   2) Test samples having 0.3≤ S/P ratio <0.4 are Suspects.
   3) Test samples having <0.3 S/P ratio negative.

| S/P value | Interpretation |
|---|---|
| S/P ≥ 0.4 | Positive |
| 0.3≤ S/P ratio <0.4 | Suspect |
| S/P < 0.3 | 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 PC :1.121, 1.201
      Mean OD = (1.121 + 1.201) / 2 = 1.161 (valid)
   3) OD of Sample : 0.831
      S/P ratio of the sample = (0.831 - 0.088 / 1.161 - 0.088) = 0.692
   4) Result interpretation: Positive

## PRECAUTIONS

1. All reagents must be allowed to come to room temperature (25±3℃) 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 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(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. 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.

**MEDIAN Diagnostics Inc.**
878, Sunhwan-daero, Dongnae-myeon, Chuncheon-si,
Gangwon-do, 24399, Republic of Korea
Tel: +82 (0)33 244 0100
Fax: +82 (0)33 244 4634
E-mail: median@mediandx.com

# QUICK PROTOCOL

**Serum Dilution (Dilution factor 1/100)**
Dilution buffer 495㎕ + Serum 5㎕

↓

**APP2 Ag Coated Plate**

↓

**Add 100㎕ of diluted Samples**
**&**
**Add 100㎕ of Undiluted PC, NC**

**25℃, 30 min** ↓ **Washing 3 times**

**Dispense 100㎕ of**
**HRPO Anti-Swine IgG Conjugate**

**25℃, 30 min** ↓ **Washing 3 times**

**Dispense 100㎕ of**
**TMB Substrate**

**25℃, 15 min** ↓

**Dispense 50㎕ of**
**Stop Solution**

↓

**Measure OD at 450nm**