# HRPro® ASFV Ab i-ELISA ver 2.0

Cat No. ES-ASF-05

## GENERAL DESCRIPTION

African swine fever (ASF) is an infectious disease of domestic and wild pigs of all breeds and ages, caused by ASF virus (ASFV). The clinical signs vary from peracute, acute, subacute to chronic, depending on the virulence of the virus. Acute disease is characterised by high fever, haemorrhages in the reticuloendothelial system, and a high lethality.

**HRPro® ASFV Ab i-ELISA ver 2.0** is designed to detect presence of anti-ASFV antibody in swine serum. The ELISA plate is coated with recombinant ASFV antigen. If ASFV specific antibodies are present in a sample, the antibodies will bind to the antigen and react with HPRO Conjugate. The complex of anti-ASFV antibody and HRPO Conjugate is detected by color development after the enzyme substrate is added. Strong color development indicates the presence of antibodies to ASFV in the sample serum. Very weak or no color development indicates the absence of antibodies to ASFV in the sample. The result is calculated by using the sample to positive control (S/P) ratio.

## COMPOSITION OF KIT

| No. | Composition | 192 Tests/Kit | 480 Tests/Kit |
|---|---|---|---|
| 1 | ASFV Antigen Coated Plate | 2 plates | 5 plates |
| 2 | 10X Washing Buffer | 120 ml x 1 | 240 ml x 1 |
| 3 | Dilution Buffer | 120 ml x 1 | 240 ml x 1 |
| 4 | HRPO Conjugate | 40 ml x 1 | 70 ml x 1 |
| 5 | Positive Control | 3 ml x 1 | 7 ml x 1 |
| 6 | Negative Control | 3 ml x 1 | 7 ml x 1 |
| 7 | TMB Substrate | 30 ml x 1 | 70 ml x 1 |
| 8 | Stop Solution | 20 ml x 1 | 40 ml x 1 |
| 9 | Sealing Film | 2 sheets | 5 sheets |
| 10 | Instruction Manual | 1 book | 1 book |

## PREPARATION

1. All reagents must be allowed to come to room temperature (25±3°C) before use. Mix reagents by gentle swirling.
2. 1X washing buffer preparation
   1) Shake 10X Washing Buffer gently.
   2) Dilute 1 part of 10X Washing Buffer with 9 parts of deionized water. The diluted 1X washing buffer is stable for 1 weeks at room temperature (25±3°C).
3. Sample preparation
   1) Serum samples may be used in this assay.
   2) Use fresh serum samples for the best result. Serum samples can be stored at 2~8°C for less than 3 days or −20°C 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) Dilute test samples 1:100 with Dilution Buffer. (e.g., by diluting 5 µl of sample with 495 µl of Dilution Buffer). Diluted Samples should be mixed well prior to dispensing into the Antigen Coated Plate.
4. Do not dilute the Positive Control and the Negative Control.
5. TMB Substrate should be warmed up for 30 minutes at room temperature (25±3°C) before use (10 ml/plate). If stored at low temperature, the color development may be poor.

## TEST PROCEDURE

1. Remove the Antigen Coated Plate from protective foil pouch.
2. Add 100 µl of the diluted serum sample to each well of the plate, keeping the wells A1 and A2 for Positive Control and the wells B1 and B2 for Negative Controls. Be careful not to spill samples from well to well.
3. Add 100 µl of undiluted Positive and Negative Controls in the designated wells.
4. Seal the plate with enclosed Sealing Film.
5. Incubate for 45 minutes at room temperature (25±3°C).
6. Wash each well 3 times with 1X washing buffer (300 µl 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 µl of HRPO Conjugate to each well.
8. Seal the plate and incubate for 30 minutes at room temperature (25±3°C).
9. Wash each well as step 6.
10. Dispense 100 µl of TMB Substrate to each well.
11. Seal the plate and incubate for 15 minutes at room temperature (25 ±3°C). Check the density of color development by naked eyes.
12. Add 50 µl of Stop Solution to each well of the plate. Shake the test plate shortly (1~5 sec). Be careful not to spill.
13. Read the plate at 450nm wavelength immediately.
14. Validate and calculate the results.

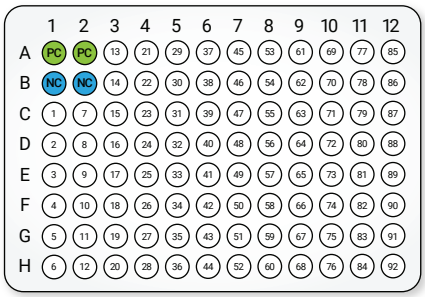


Plate template example (1-well Test)

## RESULT INTERPRETATION

1. Validate if the mean OD of the PC is higher than 0.5 and the mean OD of the NC is lower than 0.3. If these criteria are not met, the test are invalid and the samples must be retested.
2. Calculate a S/P ratio by dividing the mean OD value of a sample by the mean OD value of the Positive Control as below.

$$S/P = \frac{\text{Sample OD}}{\text{Positive Control OD}}$$

3. Result interpretation
   1) Test samples having ≥ 0.25 S/P are positive.
   2) Test samples having < 0.25 S/P are negative.

| S/P Value | Interpretation |
|---|---|
| S/P ≥ 0.25 | Positive |
| S/P < 0.25 | 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) Mean OD of Sample : 1.939
      → S/P ratio of the sample = (1.939 / 1.161) = 1.67

   **→ Result interpretation : Positive**

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## PRECAUTIONS

1. All reagents must be allowed to come to room temperature (25±3°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 with the HRPO Conjugate.
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. Be careful 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.

## QUICK PROTOCOL

1. Serum dilution (1/100)
(Dilution Buffer 495 µl
+
Serum 5 µl)

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2. Prepare ASFV Antigen Coated Plate

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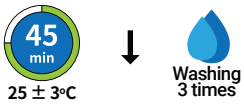
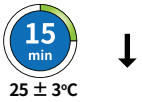
3. Dispensing Samples
(Diluted Sample 100 µl
&
Undiluted PC and NC 100 µl)




4. HRPO Conjugate 100 µl


5. TMB Substrate 100 µl




6. Stop Solution 50 µl

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7. Measure OD 450 nm

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8. Result interpretation

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