ASFV Antibody Indirect ELISA Kit

ASFV Ab Test

Product Number: E39011
Product Unit: 1 plate-96wells, 2 plates-192wells, 5 plates-480wells,
for 10plates, 2 units of 5plates will be provided

Manufacturer Information
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1. Introduction

African swine fever is a highly contagious and deadly viral disease affecting both domestic and wild pigs of all ages. ASFV is not a threat to human health and cannot be transmitted from pigs to humans. It is not a food safety issue. As a key to ASFV eradication program, antibody or antigen testing shows great significance and importance in the very beginning and in the whole process. To detect the antibody or antigen in swine serum and plasma samples, high sensitivity, reproducibility and stability are required.

2. Principle

The 96well microtiter plate was precoated with ASFV antigen. ASFV antibody exists in the serum sample, which will capture the ASFV antigen in wells and formed antigen-antibody complex. Wash away the unbound components, then add the specific enzyme-labeled antibody, which will combine with the antigen-antibody complex. Then wash away the unbound enzyme-labeled antibody, add substrate for color rendering, the measured intensity is related to the amount of antibody present in the sample.

3. Application

This ELISA kit can be used to detect ASFV specific antibody level in serum.

4. Precautions

- Store the kit at 2-8℃,Check the lot number and expiration date before use.
- Bring the test kit to room temperature before use. For example, take it out from the cold storage and put at room temperature for at least 30min.
- The stop solution in the kit is acidic, please make sure do not touch it with your hand or skin. If it is not provided in the kit, please use 0.5M sulfuric acid as stop solution.
- The component of the kit is noninfectious, but the field sample shall be treated as potentially infectious. Please handle all these materials properly according to your lab regulations.
- After experiment, all lab materials shall be handled properly according to local regulations.

5. Limitations of Test

This ELISA kit is currently designed for veterinary use. We recommend validating in your own lab with different methodologies to confirm the performance. If it is not used for the mentioned purpose, please contact us for help.

6. Reagent Provided

This kit is available in the format of 1 plate (96wells), 2 plates(192wells) and 5 plates(480wells).

Item No.	Description	1 plate	2 plates	5 plates
1	Microplate precoated with antigen	1 X 96 well	2 X 96 wells	5 X 96 wells
2	Positive Control	1 X 1 ml	1 x 1 ml	1 x 2 ml
3	Negative Control	1 X 1 ml	1 x 1 ml	1 x 2 ml
4	Enzyme Conjugate	1 X 25 ml	1 x 25 ml	1 x 60 ml

5	Sample Buffer	1 X 30 ml	2 x 30 ml	1 x 90 ml
6	25X Wash Buffer	1 X 30 ml	1 X 30 ml	1 X 60 ml
7	TMB Substrate	1 X 25 ml	1 x 25 ml	1 x 60 ml
8	Stop Solution	1 X 20 ml	1 x 20 ml	1 x 40 ml
9	Kit Instruction	1 piece	1 piece	1 piece

7. Instrument Required

- ELISA reader with 450nm
- Micropipette 20-200ul
- Micropipette Multi-Channel 50-300ul

8. Reagent and Sample Preparation

- Reagents preparation: Make sure the kit and all test samples are returned to room temperature (18-26 °C) before use. Shake each reagent gently before adding into the well.
- Wash buffer: dilute 25X wash buffer with sterial water or ionized water in the volume ratio of **1:24**, for example, 10ml 25Xwash buffer + 240ml water. This diluted wash buffer can be stored at 2-8 °C for no more than 7 days.
- Sample preparation: tTake the whole blood, centrifuge for 10 minutes at 4000 r/min, then collect the supernate. Or let the whole blood coagulate naturally at room temp. and collect the supernatant layer as serum. Dilute the serum with sample buffer in the ratio of **1:49** (5 µl serum + 245 µl sample buffer). Mix thoroughly for use.

9. Assay Procedure

1) Take the microplate from the zip-bag, and take required microwell strips and return the rest to the zip bag and store at 2-8°C.
2) **Add control**: add 100ul positive control into two wells (100ul/well). add 100ul negative control into another two wells (100ul/well).
3) **Add diluted sample**: Add 100ul diluted serum sample into the rest wells (100ul/well), and incubate at **room temperature (22-28 ^{0}C) for 30min**.
4) **Washing**: Pour the liquid out from the wells and wash with wash buffer (300ul per well, the 25X wash buffer should be diluted before use) for 5 times. Tap the residue liquid against absorbent paper to make sure the plate is dry after washing.
5) **Add enzyme conjugate**: Add 100ul enzyme conjugate into each well. Cover the plate again and then incubate at **room temperature (22-28 ^{0}C) for 30min.**
6) Repeat the washing step again.
7) **Add substrate**: add the substrate solution into each well, 100ul per well.
8) **Incubation: c**over the plate again and then incubate at **room temperature for 15min**.
9) **Add stop solution**: add 50ul stop solution into each well to stop the reaction.
10) Using ELISA reader to read the plate at 450nm.

10. Result Determination

10.1 Valid criteria of the testing:

The test results are valid only if the **Average OD value of negative control** is below **0.30**, and **average OD of positive control** must be **greater than 0.80**. Otherwise, please run the analysis again with new kit.

10.2 Calculation of S/P:

S/P = **(OD of sample – average OD of negative control) / (average OD of positive control – average OD of negative control)**

10.3 Result determination:

Positive: S/P ≥ 0.4;

Suspicious Range: 0.3 ≤ S/P < 0.4;

Negative: S/P < 0.3.

11. Performance of Test

According to field test with over 500 samples, the sensitivity of the kit is 96.8%, and the specificity of the kit is 99.7%. This kit is approved by the Ministry of Agriculture and Rural Affairs, China

12. Storage and expiration

The kit shall be store at 2-8 °C, avoid direct sunlight.
The valid period is 12 months.

13. References

(1) https://www.journals.elsevier.com/virus-research/news/african-swine-fever-virus-asfv/