Technical Manual

African Swine Fever Antibodies ELISA Kit (Competitive)

- Catalogue Code: ADES0045
- Antibody ELISA Kit
- Research Use Only

1. Test principle

This kit is comprised by HRP conjugate, other reagents and ELISA Microtiter plate pre-coated with recombinant African swine fever (ASFV) p30 and VP72 antigen. Apply the principle of enzyme-linked immunoassay (ELISA) to detect ASFV-Ab in serum of porcine. During the experiment, add control and samples into the ELISA Microtiter plate, ASFV-Ab will be bound with the antigen on the ELISA Microtiter plate. Then wash the plate to remove unbound components, horseradish peroxidase (HRP) conjugate is added to each ELISA Microtiter plate well. The unbound HRP Conjugate will be removed by washing and substrate reagent is added for color development. At last, end the reaction by adding Stop Solution to produce a yellow product. There is a negative correlation between the OD value of samples and the concentration of ASFV-Ab. Measure the absorbance value of each well by using a microplate reader with 450 nm (630 nm) wavelength, then we can judge whether ASFV antibody exist in the sample.

2. Kit components

Item	Specification
ELISA Microtiter plate	96 wells
HRP Conjugate (red)	6 mL
Sample Diluent	11 mL
20×Concentrated Wash Buffer	30 mL
Substrate Reagent	11 mL
Stop Solution	6 mL
Positive Control (green)	1 mL
Negative Control	1mL
Plate Sealer	3pieces
Sealed Bag	1 piece
Manual	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

3. Other materials required but not supplied

- Microplate Reader with 450 nm wavelength filter or dual-wavelength (450/630 nm)
- High-precision transferpettor (10μl-100μl, 100μl-1000μl), EP tubes and disposable pipette tips
- 37° C incubator or water bath
- Deionized or distilled water
- Absorbent paper

4. Notes

1. Wear gloves and work clothes during experiment, and the disinfection and isolation system should be strictly performed. All the waste should be handled as contaminant.
2. The stop solution is corrosive, it should be avoided to contact with skin and clothing. Wash immediately with plenty of water if contacted carelessly.
3. FOR RESEARCH USE ONLY. ELISA Microtiter plate should be covered by plate sealer. Avoid the kit to strong light.
4. Concentrated wash buffer at low temperature condition is easy to crystallize, it should be adjusted to room temperature in order to dissolve completely before use.
5. Each well must be filled with liquid when washing in order to prevent residual free enzyme.
6. The tested sample should keep fresh. Hemolysis, contaminated samples may lead to abnormal results.
7. The results shall depend on the readings of the microplate reader.
8. **Each reagent is optimized for use in the ADES0045. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other ADES0045 with different lot numbers.**
9. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
10. If there is a small amount of suspended solids in the positive control, negative control or HRP conjugate, it is a normal phenomenon. The positive quality control component is inactivated natural ingredients.
11. ELISA Microtiter plate should be returned to room temperature before opening the bag. Unused ELISA Microtiter plate should be repacked in sealed bags with desiccants to avoid damp. In this store condition, the unused plate should be used within one month.

5. Storage and expiry date

Store at 2-8° C. Avoid freeze.
Please store the opened plate at 2-8° C, the shelf life of the opened kit is up to 1 month.
Expiry date: expiration date is on the packing box.

6. Sample preparation

1. **Serum:** Use the conventional method to prepare serum, the serum must be clear, no hemolysis and no pollution. Samples can be conserved at 2-8° C in 1 week, and it should be stored at - 20° C for a long term storage.
2. **Wash Buffer:** The **20×Concentrated Wash Buffer** should be adjusted to room temperature to make the sediment dissolved fully before use, then dilute it with distilled or deionized water at 1:19. Ready to use Wash Buffer should be stored at -4°C for up to 1

week.

7. Assay procedure

Restore all reagents and samples to room temperature (25° C) before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2-8° C.

1. **Number:** number the sample and control in order (multiple well), and keep a record of control wells and sample wells. Set 2 wells for positive/negative control respectively. **Samples need test in duplicate.**
2. **Add sample diluent:** add 50 μL of **Sample Diluent** to each sample well.
3. **Add sample:** add 50 μL of **Positive/Negative control** to positive/negative control well, then add 50 μL **Serum** to the sample wells.
   Note: In order to ensure consistent incubation times for different samples, it is suggest to take sample, positive and negative control (not less than 50 μL) into another plate, number the sample well, then take them into the precoated ELISA Microtiter plate with multichannel pipetting. Or directly add samples.
4. **Incubate:** cover the plate sealer and mix thoroughly, incubate at 37° C for 60 min in shading light.
5. **Wash:** remove the liquid in each well. Immediately add 300 μL of **Wash Buffer** to each well and wash. Repeat wash procedure for 4 times, 30 s intervals/time. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
6. **HRP conjugate:** add 50 μL of **HRP Conjugate** into each well, cover the plate sealer and incubate at 37° C for 30 min in shading light.
7. **Wash:** Repeat step 5 for washing.
8. **Color development:** Add 100 μL of **Substrate Reagent** into each well and mix thoroughly. Cover the plate sealer and mix thoroughly, incubate at 37° C for 15 min in shading light.
9. **Stop reaction:** add 50 μL of **Stop Solution** into each well, mix thoroughly.
10. **OD measurement:** measure the absorbance value (A-value) of each well by using a Microplate Reader with 450 nm/630 nm wavelength. **Note: Read the results within 10 min.**

8. Interpretation of the results

1. OD_{NC}: Average OD of negative control;
   OD_{PC}: Average OD of positive control;
   OD_{S}: Average OD of positive sample.
2. Inhibition rate of positive control (PI_{PC})

$$PI_{PC} = （1 - \frac{OD_{PC}}{OD_{NC}}） \times 100\%$$

3. Inhibition rate of sample (PI_s)

$$PI_S = 1 - \frac{OD_S}{OD_{NC}} \times 100\%$$

4. If OD_{NC}>0.5, and PI_{PC} > 60%, the test result is valid, otherwise the test should be conduct again.
5. Positive result: PI_S > 50%
6. Negative result: PI_S ≤ 50%

9. Limitations of this test method

1. This test is only used as the qualitative detection of ASFV antibodies in serum of porcine. A rough estimate of antibody concentration (high, general, low) can be calculated based on the OD value.
2. The detection results of this kit are only for reference. For confirmation of the result, please combine the symptoms and other methods of detection, this detection cannot be used as the only criteria for result.

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Contact Details

Email: info@assaygenie.com

Web: www.assayenie.com