



Data Sheet

African Swine Fever Virus Antibody (ASFV Ab)ELISA Kit

Catalogue No.:CK-E30016

Specification: 96T;96T*2;96T*5

Storage Conditions: The kit shall be stored at 2-8 °C. Avoid moisture.

Shelf life: 12 months. Please use within 2 months after opening. The date of manufacture is presented in the label of the box.

Sample: porcine serum or plasma

Introduction:

African swine fever (ASF) is an acute, hemorrhagic, and highly contagious disease affecting domestic pigs and various wild boars, such as African and European wild boars. The World Organisation for Animal Health (WOAH) classifies it as a notifiable animal disease.

This kit is used to detect African swine fever virus (ASFV) antibodies in pig serum and can be applied to evaluate the immune efficacy of ASFV vaccines.

Instrument: microplate reader, adjustable micropipette, constant temperature device (37°C), centrifuge.

Components:

Reagent	Specification		
Microtiter Plate	96wells	96wells×2	96wells×5
Sample Diluent (yellow cap)	1×50mL	1×50mL	1×200mL
HRP conjugate (red cap)	1×11mL	2×11mL	2×26mL
Concentrated Wash Buffer (20×) (white cap)	1×40mL	1×40mL	1×200mL
Substrate Reagent A(white cap)	1×6mL	1×11mL	1×26mL
Substrate Reagent B (black cap)	1×6mL	1×11mL	1×26mL
Stop Solution (yellow cap)	1×6mL	1×11mL	1×26mL
Positive Control (red cap)	1×1.0mL	1×1.5mL	1×2.0mL
Negative Control (green cap)	1×1.0mL	1×1.5mL	1×2.0mL
Adhesive Membrane	1	2	5
Sealed bag	1	1	2
Dilution plate	1	2	5
Instruction	1	1	1

Experimental preparation

Restore all reagents and samples to room temperature (adjust to around 25°C) for more than 30 min before use.

This is a crucial step to ensure there is no precipitation in the reagents.

1. Sample Preparation: The serum should be clear, without hemolysis or contamination. Samples can be stored at 2-8°C for up to 1 week, and for long-term storage, they should be kept at -20°C.

2. Solution preparation: Dilute the concentrated wash buffer (20×) by a factor of 20 (Concentrated wash buffer/Deionized water= 1: 19). What obtained is the **working wash buffer**. *Crystallization may occur during storage. If the crystals remain after standing at room temperature for 30 minutes, dissolve them by heating and let it return to room temperature before use.*

3. Sample dilution:

Dilute the prepared serum at a 1:1 ratio with the **sample diluent** (e.g., add 100µL of sample diluent to the dilution plate, followed by the addition of 100µL of the serum, and mix well). What obtained is the **diluted sample**.

The negative and positive controls should be used directly without dilution.

Kind reminder: First, use the dilution plate to dilute all serum samples. Then, transfer the diluted serum to the Microtiter Plate to ensure consistent reaction time.

ELISA procedure

Place all reagents and samples to room temperature (adjust to around 25°C) for 30min. Gently shake the reagent bottles before use.

Take out the frame of the microplate along with the required number of wells. Then place the unused microplate wells into the sealed bag with the desiccant provided. Store the remaining kit in the refrigerator at 2-8°C.

1. Put the required number of the wells on the plate and set up 2 wells each for negative/positive control.
2. Add 300µL of **working wash buffer** to each well, no need to let it stand, then discard. Repeat the washing process 2 times. Invert the plate and tap it against a thick absorbent paper (or lint-free cloth), with a soft towel placed underneath. (Bubbles that are not removed after tapping dry can be punctured with a clean pipette tip).
3. Add 100µL of **negative control** to each negative control well. Then add 100µL of **positive control** to each positive control well. For each sample well, add 100µL of **diluted sample**.
4. Shake the plate gently by hand (or use a microplate shaker) for 10s, cover with adhesive membrane and incubate at 37°C (water bath recommended) in the dark for 1 hour.
5. Discard the liquid from the wells. Add 300µL of **working wash buffer** to each well, no need to let it stand, then discard. Repeat the washing process 5 times. Invert the plate and tap it against a thick absorbent paper (or lint-free cloth), with a soft towel placed underneath. (Bubbles that are not removed after tapping dry can be punctured with a clean pipette tip).
6. Add 100µL of **HRP conjugate** to each well, cover with adhesive membrane, and incubate at 37°C in the dark for 30 minutes.

7. Washing. Same as step 5.
8. Add 100µL of TMB substrate to each well. shake gently by hand (or use a microplate shaker) for 5s, cover with adhesive membrane, and incubate at 20-25°C in the dark for 10 minutes.
9. Add 50µL of **stop solution** to each well and shake gently by hand (or use a microplate shaker) for 5s. Read absorbance (**A value**) at 630nm with microplate reader. Finish this step within 10min.

◆ Reference Value

Under normal experimental conditions, the average A value of the negative control(A_{NC}), should be ≥ 0.6 , while the average A value of the positive control(A_{PC}), should be ≤ 0.5 .

◆ Interpretation of Test Results

$$1. \text{PI(Percentage Inhibition\%)} = \left(1 - \frac{A_S}{A_{NC}}\right) \times 100\% ,$$

If **PI** is $\geq 40\%$, it is considered positive; if **PI** is $< 40\%$, it is considered negative."

A_S —the A value of the sample;

A_{NC} —the average A value of negative controls.

Attention

- During the experiment, gloves and lab coats should be worn. Strict and comprehensive disinfection and isolation protocols should be followed. All experimental waste should be treated as infectious material.
- The stop solution is corrosive. Avoid contact with skin and clothing. If accidentally contacted, rinse immediately with a large amount of tap water.
- **When taking the microtiter plate out of a refrigerated environment, it should be brought to room temperature before opening the bag.** Unused microplate wells should be stored in the sealed bag with a desiccant.
- During washing, each well should be filled completely with liquid to prevent any residual enzyme on the well's rim from remaining unwashed.
- The samples used for testing should be kept fresh.
- The determination of test results must be based on the readings from the microplate reader.
- Components from different lot numbers must not be mixed.