abbexa®

African Swine Fever Antibody ELISA Kit

Catalog No: abx365040

Size: 96T

Range: Qualitative

Sensitivity: Qualitative

Storage: Store at 2-8°C for 6 months.

Application: The qualitative detection of ASFV-Ab in Pig serum.

Principle of the Assay: This kit is based on enzyme-linked immuno-sorbent assay technology. An antigen is pre-coated onto a 96-well plate. Controls, test samples and HRP-conjugated reagent are added to the wells and incubated. Unbound conjugates are removed using wash buffer. TMB substrate is used to quantify the HRP enzymatic reaction. After TMB substrate is added, only wells that contain sufficient ASFV-Ab will produce a blue coloured product, which then changes to yellow after adding the acidic stop solution. The intensity of the yellow colour is proportional to the ASFV-Ab amount bound on the plate. The Optical Density (OD) is measured spectrophotometrically at 450 nm in a microplate reader, from which the presence of ASFV-Ab can be determined.

Kit Components

- Pre-coated 96-Well Microplate: 12 x 8
- Wash Buffer (25X): 50 ml
- Sample Diluent Buffer: 12 ml
- Detection Reagent (100X): 0.24 ml
- Detection Reagent Diluent: 24 ml
- TMB Substrate A: 12 ml
- TMB Substrate B: 12 ml
- Stop Solution: 15 ml
- Positive Control: 1 ml
- Negative Control: 1 ml
- Plate Sealer: 3
- Hermetic Bag: 1

Materials Required But Not Provided

- 37°C incubator
- Multi and single channel pipettes and sterile pipette tips
- Squirt bottle or automated microplate washer
- 1.5 ml tubes
- Distilled water
- Absorbent filter papers
- 100 ml and 1 liter graduated cylinders
- Microplate reader (wavelength: 450 nm)
- ELISA Shaker

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Protocol

A. Sample Preparation

Analyse immediately or store samples at 2-8°C (within 24 hrs). For long term storage, aliquot and store at -20°C or -80°C. Avoid multiple freeze-thaw cycles.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 1 hr. Centrifuge at approximately 1000 × g for 20 mins. If a precipitate appears, centrifuge again. Assay immediately or aliquot and store at -20°C or -80°C.

Notes:

- **Analyse samples at a 2-fold (1/2) dilution (i.e. add 50 µl of sample to 50 µl of Sample Diluent Buffer).** Do not dilute the provided positive and negative controls.
- Store frozen samples undiluted. Once ready to analyse, thaw samples and dilute.
- Fresh samples, or recently obtained samples, are recommended to prevent protein degradation and denaturation that may lead to erroneous results.
- NaN_3 cannot be used as a test sample preservative, since it inhibits HRP.
- If a sample is not indicated in the manual's applications, a preliminary experiment to determine the suitability of the kit will be required.

B. Reagent Preparation

If crystals have formed in the concentrated solutions, equilibrate to room temperature and gently shake or swirl the tube until the crystals have completely dissolved.

Wash Buffer: Dilute the concentrated Wash Buffer 25-fold (1/25) with distilled water (e.g. add 30 ml of 25X Wash Buffer and 720 ml of distilled water to obtain 750 ml of 1X Wash Buffer).

Detection Reagent: Dilute the concentrated Detection Reagent 100-fold (1/100) with Detecton Reagent Diluent (e.g. add 0.1 ml of 100X Detection Reagent to 9.9 ml of Detection Reagent Diluent to obtain 10 ml of 1X Detection Reagent). The 1X Detection Reagent should be freshly prepared just before running the assay. Discard any unused 1X Detection Reagent after completing the assay.

C. Assay Protocol

Equilibrate the kit components and samples to room temperature prior to use. It is recommended to measure in duplicate.

1. Set 2 positive control, 2 negative control, test sample and control (zero) blank wells on the pre-coated plate respectively, and record their positions.
2. Aliquot 100 µl of negative and 100 µl positive control into the set wells. Aliquot 100 µl of Sample Diluent Buffer in the control (zero) blank well. *Add the solution to the bottom of each well without touching the side walls. Avoid foaming or bubbles.*
3. Aliquot 100 µl of appropriately diluted sample into the test sample wells. Gently tap the plate to mix, or use a microplate shaker.
4. Cover the plate with a plate sealer and incubate in the dark for 45 mins at 37°C.
5. Remove the cover and discard the liquid. Wash the plate 5 times with 1X Wash Buffer. *Fill each well completely with Wash buffer (300 µl) using a multi-channel pipette or autowasher (1-2 mins soaking period is recommended). Complete removal of liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean absorbent paper towels.*
6. Aliquot 100 µl of 1X Detection Reagent to each well (except the blank well). Cover the plate with a plate sealer and incubate in the dark for 30 mins at 37°C.
7. Remove the cover, discard the liquid, and repeat the wash process as described above, 5 times.

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8. Aliquot 50 µl of TMB Substrate A and 50 µl of TMB Substrate B into each well. Cover the plate with the plate sealer and gently tap the plate to mix thoroughly. Incubate at 25°C for 10-15 mins. The incubation time is for reference only, the optimal time should be determined by end user. Avoid exposure to light.
9. Aliquot 50 µl of Stop Solution into each well. It is important that the Stop Solution is mixed quickly and uniformly throughout the microplate to inactivate the enzyme completely.
10. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the OD at 450 nm immediately.
11. OPTIONAL: measure the absorbance at 630 nm and subtract these values from the 450 nm values for analysis. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles.

Data Analysis:

- Mean OD of the Positive Control should be ≥ 0.8.
- [Mean OD of the Positive Control] / [Mean OD of the Negative Control] should be ≥ 4.

Calculate $\mathbf{S/P}$:

$$\mathbf{S/P} = \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Negative\ Control}}}{\mathbf{OD_{Positive\ Control}} - \mathbf{OD_{Negative\ Control}}}$$

where

$\mathbf{OD_{Sample}}$	average absorbance of sample
$\mathbf{OD_{Positive\ Control}}$	average absorbance of positive control
$\mathbf{OD_{Negative\ Control}}$	average absorbance of negative control

- Positive result: S/P ≥ 0.15
- Negative result: S/P < 0.15

Precautions:

- Before using the kit, centrifuge the tubes to bring down the contents trapped in the lid.
- Do not leave the wells uncovered for extended periods between incubations. The addition of reagents for each step should not exceed 10 mins.
- Ensure that the plate is properly sealed or covered during the incubation steps, and that the time and temperature are controlled.
- Do not reuse pipette tips and tubes.
- Do not use expired components, or components from a different kit.
- The TMB substrate should be used under sterile conditions, and light exposure should be minimised. Unused substrate should be colorless, or a very light yellow in appearance. Do not discard any residual solution back into the vial.
- Please note that this kit is optimised for detection of native samples, rather than recombinant proteins or synthetic chemicals. We are unable to guarantee detection of recombinant proteins, as they may have different sequences or tertiary structures to the native protein.
- This kit is for research use only and the results are for reference only. It is recommended to use this kit in conjunction with another detection method.

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