Version 10-04/24

User's Manual

ASFV Antibody (P72) Blocking ELISA kit

REF **DEIA-NS2309-2**

Σ **192T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

In vitro quantitative determination of COL3 concentrations in serum, plasma, cell culture supernatant and other biological samples.

Principles of Testing

This kit uses the African swine fever virus P72 antigen protein to coat the microtiter plate. In the test, negative and positive controls and diluted serum to be tested are added, and after incubation, if the sample contains African swine fever virus antibody, it will bind to the P72 protein coated on the microtiter plate. After washing to remove unbound antibodies and other components, add the enzyme-labeled antibody working solution, which can specifically bind to the unbound P72 antigen site on the enzyme-labeled plate. After washing to remove unbound enzyme-labeled antibody, add substrate solution to the well, react with the enzyme to form a blue product, add the stop solution, the color changes under the action of peroxidase, and use a microplate reader at 450nm to measure each The OD value in the reaction well, the depth of the solution color is inversely proportional to the antibody concentration in the sample to be tested.

Reagents And Materials Provided

1. P72 Microplates: 96-well microtitration plates. 2 plates
2. Positive Control: 500 µl, positive serum. Store this reagent between 2°C - 8°C.
3. Negative Control: 500 µl, negative serum. Store this reagent between 2°C - 8°C.
4. 25×PBST: 30 mL, Store this reagent between 2°C - 8°C.
5. Sample Dilution: 12 ml, 2°C - 8°C.
6. Enzyme-labeled antibody: 12 ml, 2°C - 8°C.
7. Substrate solution: 12 ml, the chromogen tetramethylbenzidine (TMB). Store between 2°C - 8°C protected from light. This solution is ready to use.
8. Stop Solution: 12 ml.

Take the Elisa kit from the fridge around 20 minutes earlier and equilibrate to room temperature(18-25°C) befor use.

Materials Required But Not Supplied

Dilution plates, micropipettes, disposable tips, dosing tanks, deionized water, incubators, microplate readers, absorbent paper, etc.

Storage

2-8°C, 12 months

Specimen Collection And Preparation

Dilute serum samples 1:4 in Sample Dilution (for example: 12µl sample + 48µl sample diluent), negative and positive controls do not need to be diluted. Each sample should be mixed before adding to the microplate reaction plate.

Reagent Preparation

Washing Buffer:

Calculate required total volume of the washing buffer: Dilute 25× PBST 1:24 in deionized water (for example: 10mL 25× PBST + 240mL deionized water). The washing buffer can be stored at 2-8°C about 7 days.

Assay Procedure

If you donot need to use all the strips at once, place the unused unwashed strips back in the plate packet and store at 4°C. Be sure the packet is sealed with the desiccant inside.

1. Take out the enzyme-labeled reaction plate and record the position of the sample on the recording sheet. If you only need to use part of the slats, remove the required slats for testing, put the remaining slats into a ziplock bag with a desiccant, seal it and store it at 2-8°C.
2. Transfer 50µl of the diluted sample to the enzyme labeling reaction plate. Add two wells for the negative and positive controls respectively, seal the plate with a sealing film and incubate at 37°C for 30 minutes.
3. Discard the liquid from each well into a waste container. Wash the wells with approximately 300 µl of 1× Wash Buffer 5 times. After the last wash was discarded, the remaining wash in the wells was patted dry on absorbent paper.
4. Add 50µl enzyme-labeled antibody working solution to each well, seal the plate with a sealing film and incubate at 37°C for 30 minutes.
5. Repeat step 3.
6. Add 50µl of substrate solution to each well and incubate at 37°C for 12 minutes in the dark.
7. Add 50µl of stop solution to each well, and read the OD value on a microplate reader at a wavelength of 450nm.

Calculation

Calculate the average OD450nm of the negative control wells (OD_{NC}) and the average OD450nm of the positive control wells (OD_{PC}) respectively.

Validity:

Negative control OD450nm mean value (OD_{PC}) ≥ 1.0, and positive control PI value ≥ 80%.

$$PI(\%) = 100 - \frac{OD_{sample}}{OD_{NC}} \times 100$$

Interpretation Of Results

Positive Result	Equivocol Result	Negative Result
PI≥50%	40%≤PI < 50%	PI < 40%

When the sample to be tested is judged to be "equivocol", it needs to be tested again. If the PI value of the second test result is ≥ 40%, it is judged as positive, otherwise it is negative.

Precautions

1. Read the instructions carefully, and keep all reagents as required.
2. Return all reagents to room temperature (20-25°C) before use.
3. Strictly follow the operation steps to get the best results. Careful pipetting, timing, and washing of plates throughout the process is essential for precision and accuracy. Use separate tips for each sample and control.
4. Avoid moisture or water after unpacking the microtiter plate.
5. Do not expose the substrate solution to strong light or contact with oxidants, and use a clean vessel to hold the substrate solution.
6. It is forbidden to mix different batches of reagents and use expired kits.
7. Parafilm is for one-time use only to avoid cross-contamination.