Creative Diagnostics®

Version 10-04/24

User's Manual

African Swine fever Virus ELISA Antibody (P54) Test Kit

REF **DEIA-NS2309-4**

Σ **96T**

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

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

In vitro qualitative determination of ASFV Antibody (P54) in serum sample.

Principles of Testing

This kit uses the African swine fever virus P54 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 P54 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 P54 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. Microplates: 96-well microtitration plates. 1 plate
2. Positive Control: 1 ml, positive serum. Store this reagent between 2°C - 8°C.
3. Negative Control: 1 ml, negative serum. Store this reagent between 2°C - 8°C.
4. 10× Wash Buffer: 50 mL, Store this reagent between 2°C - 8°C.
5. Sample Dilution: 40 ml, 2°C - 8°C.
6. Enzyme-labeled Antibody 100×: 150 µl, 2°C - 8°C.
7. Enzyme-labeled Antibody Dilution: 11 ml
8. Substrate solution: 11 ml, Store between 2°C - 8°C protected from light. This solution is ready to use.
9. Stop Solution: 6 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, double distilled water, incubators, microplate readers, absorbent paper, etc.

Storage

2-8°C, 9 months

Specimen Collection And Preparation

Dilute serum samples 1:199 in Sample Dilution (1µl sample + 199µl Sample Dilution).

Reagent Preparation

1× Washing Buffer:

Calculate required total volume of the washing buffer: Dilute 10× Wash Buffer 1:9 in deionized water (for example: 10mL 10× Wash Buffer + 90mL double distilled water). The washing buffer can be stored at 2-8°C about 7 days.

1× Enzyme-labeled Antibody:

Calculate required total volume of the 1× Enzyme-labeled Antibody: Dilute 100× Enzyme-labeled Antibody 1:99 in Enzyme-labeled Antibody Dilution (for example: 10 µl 100× Enzyme-labeled Antibody + 990 µL Enzyme-labeled Antibody Dilution). Prepare new working solution for each use.

Assay Procedure

1. Number the sample and control in order (multiple well), and keep a record of control wells and sample wells. Set 2 wells for negative/positive control respectively.

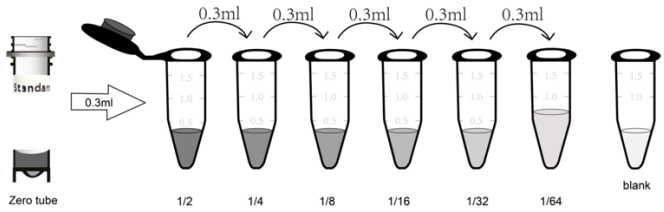
No.	Item	Size(96T)	Storage Condition for Opened Kit
E001	ELISA Microplate(Dismountable)	8×12	Put the rest strips into a sealed foil bag with the desiccant. Stored for 1 month at 2-8°C; Stored for 6 month at -20°C
E002	Lyophilized Standard	2vial	Put the rest standards into a desiccant bag. Stored for 1 month at 2-8°C; Stored for 6 month at -20°C
E003	Biotin-labeled Antibody(Concentrated, 100X)	120ul	2-8°C (Avoid Direct Light)
E034	HRP-Streptavidin Conjugate(SABC, 100X)	120ul	
E024	TMB Substrate	10ml	
E039	Sample Dilution Buffer	20ml	2-8°C
E040	Antibody Dilution Buffer	10ml	
E049	SABC Dilution Buffer	10ml	
E026	Stop Solution	10ml	
E038	Wash Buffer(25X)	30ml	
E006	Plate Sealer	5 pieces	
E007	Product Description	1 copy	

Note: "P" is positive control serum; "N" is negative control serum; the rest are serum samples to be tested

2. Incubate for 30 minutes in a 37°C incubator.
3. Discard the liquid in the wells, add 300 µl of 1× Wash Buffer to each well, wash 4 times, and pat dry each time.
4. Add 100 µl of 1× Enzyme-labeled Antibody to each well.
5. Incubate for 45 minutes in a 37°C incubator.
6. Repeat step 3 for washing.

7. Add 100 µl of Substrate solution to each well. Incubate at 37°C for 10 min in shading light.
8. Add 50 µL of Stop Solution into each well, mix thoroughly. Measure the absorbance value of each well by using a Microplate Reader with 450 nm wavelength. The reading should be completed within 5 minutes of adding the stop solution.

Calculation



Prepare standard solutions

$OD_{Negative\ control}$: Average OD450nm of the negative control

$OD_{Positive\ control}$: Average OD450nm of the positive control

Interpretation Of Results

Validity:

If the positive control serum OD450nm values are all >0.6, and the negative control serum OD450nm values are all <0.15, then the test is judged to be established.

Results:

S/P≥0.45, Positive result

S/P < 0.45, Negative result

Precautions

(1) The kit should be transported and stored at 2-8°C.

(2) During storage, all slats must be sealed with parafilm to prevent moisture from damaging the coated board. Otherwise, it must not be used.

(3) Do not expose the substrate solution to strong light and oxides. After all reagents are taken out, they must not be put back into the bottle.

(4) Read the instructions carefully.

(5) Do not use expired components or mix reagents from different batches.

(6) If crystals are found in the 10 × Wash buffer, put them at 37°C to dissolve them before use.

(7) Pay attention to the process of adding samples and washing to ensure the accuracy of the test, and it is strictly forbidden to use the mouth to absorb liquid.

(8) Do not use the serum to be tested when it is corrupted.

(9) The utensils used for inspection must be clean and avoid contact with metal utensils during the operation.

(10) The operation should be carried out in strict accordance with the instructions of the kit, and the time and temperature specified in each operation step should be strictly followed.

(11) Gloves are used during the operation, and the stop solution is corrosive, so be careful when using it. All waste liquids are treated and purified.

Tel: 1-631-624-4882 (USA)
Tel: 44-161-818-6441 (Europe)
Fax: 1-631-938-8221
Email: info@creative-diagnostics.com