Version 29-03/25

User's Manual

African Swine fever Antibodies ELISA Kit

REF **DEIA-NS2309-3**

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

This indirect ELISA detects anti-African Swine Fever virus (ASFV) antibodies in porcine serum, plasma, meat juice and blood filter paper samples.

Principles of Testing

Microwells are coated with p32, p62 and p72 ASFV recombinant proteins. Samples to be tested and controls are added to the microwells. Anti-ASFV antibodies, if present, form an antigen-antibody complex. After washing, an anti-multi-species horseradish peroxidase (HRP) conjugate is added to the wells. It fixes to the antibodies, forming an antigenantibody-conjugate-HRP complex. After elimination of the excess conjugate by washing, the substrate solution (TMB) is added. The resulting coloration is proportional to the quantity of specific antibodies present in the sample:

1. In the presence of antibodies, a blue coloration appears which becomes yellow after addition of the stop solution.
2. In the absence of antibodies, no coloration appears.

The microplate is read at 450 nm.

Note: This kit does not contain infectious material.

Reagents And Materials Provided

Microplates coated with p32, p62 and p72 ASFV recombinant proteins

Concentrated Conjugate (10X)

Positive Control

Negative Control

Dilution Buffer 14

Dilution Buffer 3

Wash Concentrate (20X)

Substrate Solution

Stop Solution (0,5 M)

Materials Required But Not Supplied

1. Mono or multi-channel micropipettors capable of delivering volumes of 10 µl, 100 µl, and 500 µl.
2. Disposable tips.
3. 96-well pre-dilution microplate.
4. Distilled or deionized water.
5. Manual or automatic wash system.

6. 96-well microplate reader.

Storage

1. The conjugate, the controls and the substrate solution must be stored at 5°C (± 3°C).
2. Other reagents can be stored between +2°C and +26°C.

Specimen Collection And Preparation

In order to avoid differences in incubation times between specimens, it is possible to prepare a 96-well plate containing the test and control specimens, before transferring them into an ELISA microplate using a multichannel pipette.

Reagent Preparation

If necessary, bring the Wash Concentrate (20×) to room temperature and mix thoroughly to ensure that the Wash Concentrate (20X) is completely solubilized. Prepare the Wash Solution (1×) by diluting the Wash Concentrate (20×) to 1:20 in distilled/deionized water. The quality of the wash step may influence results.

Assay Procedure

Allow all reagents to come to room temperature (21°C ±5°C) before use. Homogenize all reagents by inversion orvortexing.

Serum and plasma samples

1. Add:

   190 µl of Dilution Buffer 14 to each well.

   10 µl of the Negative Control to wells A1 and B1.

   10 µl of the Positive Control to wells C1 and D1.

   10 µl of each sample to be tested to the remaining wells.
2. Cover the plate and incubate 45 min ± 4 min at 21°C (± 5°C)

Filter paper samples (Whatman #1 or #3)

1. Place 2 filter paper discs (6mm) per animal in a tube or a deepwell plate.
2. Add 200 ul of Dilution Buffer 14.
3. Homogenize by agitation or vortex. Ensure that each disc is completely immersed in Dilution buffer 14. Seal each tube.
4. Elute overnight (16-20 hours) at 21°C (±5°C).
5. Homogenize by agitation, or vortex at the end of the elution.
6. Add:

   190 µl of Dilution Buffer 14 and 10 µl of the Negative Control to wells A1 and B1.

   190 µl of Dilution Buffer 14 and 10 µl of the Positive Control to wells C1 and D1.

50 µl of each filter paper sample eluate to be tested in the remaining wells.

7. Cover the plate and incubate 45 min ± 4 min at 21°C (± 5°C).

Meat Juice (suggested protocol, for Research Use Only)

Meat juice samples should be as clean as possible.

Remove debris and lipids from the sample when pipetting

1. Add:

   190 µl of Dilution Buffer 14 and 10 µl of the Negative Control to wells A1 and B1.

   190 µl of Dilution Buffer 14 and 10 µl of the Positive Control to wells C1 and D1.

   50 µl of Dilution Buffer 14 and 50 µl of each sample to be tested to the remaining wells.

2. Cover the plate and incubate 45 min ± 4 min at 21°C (±5°C).

Remaining steps common to serum, plasma filter paper samples, or meat juice (RUO)

1. Empty the wells. Wash each well 3 times with at least 300 µl of the Wash Solution. Avoid drying of the wells between washes.
2. Prepare the Conjugate 1× by diluting the Concentrated conjugate 10× to 1:10 in Dilution Buffer 3.
3. Add 100 µl of the Conjugate 1× to each well.
4. Cover the plate and incubate 30 min ± 3 min at 21°°C(±5°C).
5. Empty the wells. Wash each well 3 times with at least 300 ul of the Wash Solution. Avoid drying of the wells between washes.
6. Add 100 ul of the Substrate Solution to each well.
7. Cover the plate and incubate 15 min ± 2 min at 21°C(±5°C) in the dark.
8. Add 100 ul of the Stop Solution to each well in the same order as in step No. 6, to stop the reaction.
9. Read and record the O.D. at 450 nm.

Quality Control

The test is validated if: the mean value of the Positive Control OD(OD_{PC}) is greater than 0.350.

OD_{PC}0.350

The ratio of the mean values of the Positive and Negative Control ODs (OD_{PC} and OD_{NC}) is greater than 3.

OD_{PC}/OD_{NC} > 3

Interpretation Of Results

For each sample, calculate the S/P percentage (S/P%):

$$\mathbf{S/P\,\%} = \frac{\mathbf{OD_{sample} - OD_{NC}}}{\mathbf{OD_{PC} - OD_{NC}}} \mathbf{\times 100}$$

For all sample types (serum, plasma, filter paper or meat juice):

S/P % ≤ 30%: **NEGATIVE**

30% < S/P % < 40%: **DOUBTFUL**

S/P % ≥ 40%: **POSITIVE**

Precautions

1. Do not pipette by mouth.
2. Contains components that can be harmful to the skin and eyes and may cause sensitisation by skin contact. Avoid contact with skin and eyes. Use protective lab coat, one-way gloves and safetyglasses. The stop solution (0,5 M acid) may be harmful if swallowed.
3. Do not expose the substrate solution to bright light nor to oxidizing agents.
4. All waste should be properly decontaminated prior to disposal. Dispose in accordance with local regulations.