General Information

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

Description and Principle

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 antigen-antibody-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:

- in the presence of antibodies, a blue coloration appears which becomes yellow after addition of the stop solution.
- in the absence of antibodies, no coloration appears.

The microplate is read at 450 nm.

Note: This kit does not contain infectious material.

Kit Components

Reagents*
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)

**Quantities supplied are indicated on the kit label.*

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.
3. For detailed storage conditions of opened and/or diluted components, please refer to https://www.id-vet.com/fr/support/faq.
4. Wash and stop solutions can be used for the entire IDvet product range. Substrate solutions and dilution buffers with same batch numbers are interchangeable.

Materials required but not provided

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.

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 safety glasses. 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.

Please refer to the Material Safety Data Sheet, available upon request at info@innovative-diagnostics.com, for more detailed information.

Sample 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.

Wash Solution Preparation

If necessary, bring the Wash Concentrate **(20X)** to room temperature and mix thoroughly to ensure that the Wash Concentrate **(20X)** is completely solubilized.

Prepare the Wash Solution **(1X)** by diluting the Wash Concentrate **(20X)** to 1:20 in distilled/deionized water.

The quality of the wash step may influence results. Ensure that wells are completely empty between washes. If using an automatic washer, it is extremely important to correctly parameter the machine (mode, type of aspiration, aspiration height). For more information, please consult the "IDvet Washing Guide", available upon request.

Testing Procedure

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

Serum and plasma samples

1. Add:
   - 190 µl of **Dilution Buffer 14** to each well.
   - 10 µl of the **Negative Control** to wells A1 andB1.
   - 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 (Ø 6mm) per animal in a tube or a deepwell plate. Contact Innovative Diagnostics for more information.
2. Add 200 µl 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 1X** by diluting the **Concentrated conjugate 10X** to 1:10 in **Dilution Buffer 3.**
3. Add 100 µl of the **Conjugate 1X** 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 µl of the **Wash Solution.** Avoid drying of the wells between washes.
6. Add 100 µl 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 µl 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.

Validation

The test is validated if:

✓ the mean value of the Positive Control OD (OD_{PC}) is greater than 0.350.

$$\mathbf{OD_{PC} > 0.350}$$

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

$$\mathbf{OD_{PC}/OD_{NC} > 3}$$

Interpretation

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

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

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

Result	Status
S/P % ≤ 30%	NEGATIVE
30% < S/P % < 40%	DOUBTFUL
S/P % ≥ 40%	POSITIVE

Note: The IDSoft™ data analysis program is available free-of-charge. Please contact, for more information, support.software@innovative-diagnostics.com.

This software program can calculate many parameters (validation criteria, S/P or S/N values, titers, vaccination age, groups) and offers a graphic representation of the serological profiles of the animals tested)

Certified management system

ID Screen®
African Swine Fever Indirect
Screening Test

Indirect ELISA for the detection of antibodies against ASFV in serum, plasma, meat juice or blood filter paper samples from porcines

For *in vitro* use

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ASFS ver 0115 EN

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