General Information

This indirect ELISA diagnostic kit is designed to detect antibodies directed against the nucleoprotein (NP) of the Influenza A virus in swine serum or plasma.

It is a quantitative test designed for disease diagnosis and monitoring of vaccination with conventional vaccines.

Description and Principle

Microwells are coated with purified NP antigen.

Samples to be tested and controls are added to the wells. Anti-NP antibodies, if present, form an antigen-antibody complex.

After washing, an anti-pig horseradish peroxidase (HRP) conjugate is added to the wells. It fixes to the sample 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 depends on the quantity of specific antibodies present in the specimen to be tested:

- in the presence of antibodies, a blue solution 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.

Kit Components

Reagents*
Microplates coated with purified NP antigen
Positive Control
Negative Control
Concentrated Conjugate (10X)
Dilution Buffer 2
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. Wash, substrate and stop solutions can be used for the entire IDvet product range. Dilution buffers with same batch numbers are interchangeable.

Materials required but not provided

1. Mono or multi-channel pipettes capable of delivering volumes of 5 µl, 10 µl, 100 µl, and 300 µl.
2. Disposable tips.
3. 96 well pre-dilution plate
4. Distilled or deionized water.
5. Manual or automatic wash system.
6. 96-well microplate reader.

Precautions

1. Do not pipette by mouth.
2. The substrate solution can be irritating to the skin.
3. The stop solution (0,5M) may be harmful if swallowed. It may cause sensitisation by skin contact (**R22-43**). Avoid contact with skin (**S24-37**).
4. Do not expose the substrate solution to bright light nor to oxidizing agents.
5. All waste should be properly decontaminated prior to disposal. Dispose in accordance with local regulations.

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 at info@id-vet.com.

Testing Procedure

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

Samples are tested at a final dilution of 1:100 in **Dilution Buffer 2.**

1. In a pre-dilution plate, add:
   - 5 µl of the **Negative Control** to wells A1 and B1,
   - 5 µl of the **Positive Control** to wells C1 and D1,
   - 5 µl of each sample to be tested,
   - 245 µl of **Dilution Buffer 2** to each well.

Note: It is recommended to respect the indicated order of deposit to be able to visually control addition of sample to each well.

2. In the ELISA microplate, add:
   - 50 µl of **Dilution Buffer 2**.
   - 50 µl of the **pre-diluted controls and samples** as prepared above.
3. Cover the plate and incubate **1 hour** ± **6 min** at 37°C (± 2°C).
4. Prepare the **Conjugate 1X** by diluting the **Concentrated conjugate 10X** to 1:10 in **Dilution Buffer 3.**
5. Empty the wells. Wash each well 3 times with at least 300 µl of the **Wash Solution 1X.** Avoid drying of the wells between washes.
6. Add 100 µl of the **Conjugate 1X** to each well.
7. Cover the plate and incubate **30 min** ± **3 min** at 21°C (± 5°C).
8. Empty the wells. Wash each well 3 times with at least 300 µl of **Wash Solution 1X.** Avoid drying of the wells between washes.
9. Add 100 µl of the **Substrate Solution** to each well.
10. Cover the plate and incubate **15 min** ± **2 min** at 21°C (± 5°C) in the dark.
11. Add 100 µl of the **Stop Solution** to each well, in the same order as in step N°8, to stop the reaction.
12. Read and record the O.D. at 450 nm.

Validation

The test is validated if:

✓ the mean OD value of the Positive Control (OD_{PC}) is greater than 0.250.

$$\mathbf{OD_{PC} > 0.250}$$

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

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

Interpretation

For each sample, calculate the S/P ratio and antibody titer as follows:

1) S/P ratio

$$\mathbf{S/P = \frac{OD_{sample} - OD_{NC}}{OD_{PC} - OD_{NC}}}$$

2) Antibody titer

$$\mathbf{\log_{10}(titer) = 1.2 \times \log_{10}\left(S/P\right) + 3.500}$$

$$\mathbf{titer = 10^{\log_{10}(titer)}}$$

Results are interpreted as follows:

S/P value	ELISA antibody titer	Immune status
S/P ≤ 0.4	TITER ≤ 1053	Negative
S/P > 0.4	TITER > 1053	Positive

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

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

IDvet Innovative Diagnostics

Certified management system

ID Screen®
Influenza A Nucleoprotein Swine Indirect

Indirect ELISA for the detection of antibodies against the Influenza A virus nucleoprotein in swine serum or plasma

For *in vitro* use

FLUNPS-SW ver 0517 GB

007647

IDvet, 310, rue Louis Pasteur – Grabels - FRANCE
Tel:+ 33 (0)4 67 41 49 33 - Fax: + 33 (0)4 67 45 36 95
www.id-vet.com - E-mail: info@id-vet.com