

General Information

This diagnostic kit is designed to detect antibodies directed against the internal nucleocapsid of the Influenza A virus.

It can be used with pigs, horses or birds serum or plasma but also with porcine oral fluid.

Description and Principle

Wells are coated with Influenza A nucleoprotein (NP).

Specimens to be tested and controls are added to the microwells. Anti-NP antibodies, if present, form an antibody-antigen complex which masks the NP epitopes.

An anti-NP-peroxidase (HRP) conjugate is added to the microwells. It fixes to the remaining free NP epitopes, forming an antigen-conjugate-HRP complex.

After washing in order to eliminate the excess conjugate, 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 absence of antibodies, a blue solution appears which becomes yellow after addition of the stop solution.
- in the presence of antibodies, no coloration appears.

The microplate is read at 450 nm.

Kit Components

Reagents*
Microplates coated with the NP
Concentrated Conjugate (10X)
Positive Control
Negative Control
Dilution Buffer 3
Dilution Buffer 2
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 ($\pm 3^\circ\text{C}$).
2. The 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.

Note: If needed, IDvet can supply you with additional volumes of the above components.

Materials required but not provided

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

Precautions

1. Do not pipette by mouth.
2. The substrate solution can be irritating to the skin.
3. The stop solution (0.5 M) 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.

Wash Solution Preparation

If necessary, bring the Wash Concentrate (**20X**) to room temperature and mix thoroughly to ensure that the Wash Concentrate is completely solubilized. Prepare the Wash Solution (**1X**) by diluting the Wash Concentrate (**20X**) in distilled/deionised 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 the reagents to come to room temperature before use. Homogenize all reagents by inversion or vortexing.

Note 1: Protocols for chicken, turkey, quail, guinea fowl, horse, duck, ostrich, and swine samples are proposed. Please consult IDvet for protocols for other species.

1. Samples from different species require different dilution factors.

🔥 For chicken, turkey, quail, guinea fowl, duck, geese, and ostrich serum or plasma samples, add:

- 40 µl of **Dilution Buffer 2** in each well.
- 10 µl of the **Positive Control** to wells A1 and B1.
- 10 µl of the **Negative Control** to wells C1 and D1.
- 10 µl of each sample to be tested to the remaining wells.

For horse serum or plasma samples, add:

- 90 µl of **Dilution Buffer 2** in each well.
- 10 µl of the **Positive Control** to wells A1 and B1.
- 10 µl of the **Negative Control** to wells C1 and D1.
- 10 µl of each sample to be tested to the remaining wells.

For swine serum or plasma samples, add:

- 90 µl of **Dilution Buffer 2** and 10µl of the **Positive Control** to wells A1 and B1.
- 90 µl of **Dilution Buffer 2** and 10µl of the **Negative Control** to wells C1 and D1.
- 200 µl of **Dilution Buffer 2** and 5 µl of each sample to be tested in the remaining wells.

🔥 For swine oral fluid samples, add:

- 90 µl of **Dilution Buffer 2** and 10µl of the **Positive Control** to wells A1 and B1.
 - 90 µl of **Dilution Buffer 2** and 10µl of the **Negative Control** to wells C1 and D1.
 - 50 µl of **Dilution Buffer 2** and 50 µl of each sample to be tested in the remaining wells.
2. Cover the plate and incubate **60 min \pm 6 min at 37°C ($\pm 2^\circ\text{C}$).**
 3. Empty the wells. Wash each well **5** times with approximately 300 µl of **Wash Solution**. Avoid drying of the wells between washes.
 4. Prepare the **Conjugate 1X** by diluting the **Concentrated Conjugate 10X** to 1/10 in **Dilution Buffer 3**.
 5. Add 50 µl of the **Conjugate 1X** to each well.
 6. Cover the plate and incubate **30 min \pm 3 min at 21°C ($\pm 5^\circ\text{C}$).**
 7. Empty the wells. Wash each well 3 times with approximately 300 µl of **Wash Solution**. Avoid drying of the wells between washes.
 8. Add 50 µl of the **Substrate Solution** to each well.
 9. Cover the plate and incubate **10 min \pm 1 min at 21°C ($\pm 5^\circ\text{C}$) in the dark.**
 10. Add 50 µl of the **Stop Solution** to each well in order to stop the reaction. The stop solution should be added in the same order as in step 8.
 11. Read and record the O.D. at 450 nm.

Validation

The test is validated if:

- ✓ the mean value of the Negative Control O.D. (OD_{NC}) is greater than 0.700.

$$OD_{NC} > 0.700$$

- ✓ the mean value of the Positive Control (OD_{PC}) is less than 30 % of the OD_{NC}.

$$OD_{PC} / OD_{NC} < 0.3$$

Interpretation

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

$$S/N \% = OD_{\text{sample}} / OD_{NC} \times 100$$

Samples presenting a S/N %:

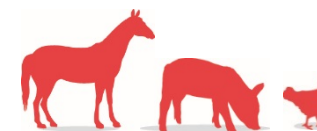
- greater than or equal to 50 % are considered negative.
- between 45% and 50% are considered doubtful.
- less than or equal to 45% are considered positive.

Result	Status
S/N % ≤ 45 %	POSITIVE
45 % < S/N % < 50 %	DOUBTFUL
S/N % ≥ 50 %	NEGATIVE

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 or S/N values, titers, vaccination age, groups) and offers a graphic representation of the serological profiles of the animals tested).

ID Screen® Influenza A Antibody Competition Multi-Species



Competitive ELISA for the detection of antibodies against the nucleoprotein of the Influenza A virus in avian, porcine, or equine serum, plasma or porcine oral fluid.

For *in vitro* use

September 2017:

- » Change in sample dilution factor for several species

December 2016:

- » Addition of a new sample type: porcine oral fluid

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