General Information

This diagnostic kit is designed to specifically detect antibodies directed against the H5 antigen of the Influenza A virus in bird sera (e.g.: chicken, duck, turkey or other susceptible species).

Description and Principle

Wells are coated with the haemagglutinin H5.

Specimens to be tested and controls are added to the microwells. Anti-H5 antibodies, if present, form an antibody-antigen complex which masks the H5 epitopes.

An anti-H5-peroxidase (HRP) conjugate is added to the microwells. It fixes to the remaining free H5 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 Ag H5
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. 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.

Materials required but not provided

1. Mono or multi-channel pipettes 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.

Samples 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 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 **(**21°C ± 5°C) before use. Homogenize all reagents by inversion or vortexing.

1. Add:
   - 25 µl of **Dilution Buffer 14** to each well.
   - 25 µl of the **Positive Control** to wells A1 and B1.
   - 25 µl of the **Negative Control** to wells C1 and D1.
   - 25 µl of each sample to be tested to the remaining wells.
2. Cover the plate and incubate **1 h** ± **5 min** at 37°C (± 2°C).
3. Empty the wells. Wash each well 3 times with approximately 300 µl of the **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 100 µl of the **Conjugate 1X** to each well.
6. Cover the plate and incubate **30 min** ± **2 min** at 21°C (± 5°C).
7. Empty the wells. Wash each well 3 times with approximately 300 µl of the **Wash Solution.** Avoid drying of the wells between washes.
8. Add 100 µl of the **Substrate Solution** to each well.
9. Cover the plate and incubate **15 min** ± **1 min** at 21°C (± 5°C) in the dark.
10. Add 100 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 N°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 %):

$$S/N\% = \frac{OD_{sample}}{OD_{NC}} x100$$

Samples presenting a S/N%:

- greater than or equal to 60% are considered negative.
- between 50% and 60% are considered doubtful.
- less than or equal to 50% are considered positive.

Result	Status
S/N % ≤ 50**%**	POSITIVE
50**%** < S/N % < 60%	DOUBTFUL
S/N % ≥ 60 **%**	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).

IDvet Innovative Diagnostics

Certified management system

ID Screen®
Influenza H5 Antibody Competition

Competitive ELISA for the detection of antibodies against the hemagglutinin H5 of the Avian Influenza virus in avian serum

For *in vitro* use

February 2015:

Standardization of avian range testing procedures

- **Conjugate now based on a new monoclonal antibody**
- **Dilute samples and controls in Dilution buffer 14 (instead of Dilution buffer 11)**
- **Sample and control dilution factor 1:2 (instead of 1:5)**
- **Add 100 µl of 1X Conjugate, Substrate solution and Stop solution (instead of 50 µl previously)**
- **Incubate Substrate solution for 15 min (instead of 10 min).**
- **Cut-off of 50-60% instead of 35%-40%**

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