General Information

This diagnostic kit is designed to specifically detect antibodies directed against the N1 Antigen of the Influenza virus.

It can be used with bird sera.

Description and Principle

The wells are coated with purified Influenza N1 antigen.

Specimens to be tested and the controls are added to the microwells. Anti-N1 antibodies, if present, form an antibody-antigen complex which masks the N1 epitopes.

An anti-N1-peroxidase (HRP) conjugate is added to the microwells. It fixes to the remaining free N1 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 N1 Antigen
Concentrated Conjugate (10X)
Positive Control
Negative Control
Dilution Buffer 14
Dilution Buffer 3
Wash Concentrate (20X)
Substrate Solution (TMB)
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. Components bearing the same name (*wash solution, dilution buffers*) can be used in the entire IDvet product range.

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

Materials required but not provided

1. Mono or multi-channel micropipettors capable of delivering volumes of 10 µl, 100 µl, and 200 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 single-use material used for the assays should be decontaminated by immersion in freshly prepared 5% sodium hypochlorite for minimum 1 hour before elimination, or by autoclaving at 120°C.

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/deionized water.

Testing Procedure

Allow all the reagents to come to room temperature before use. Homogenize all reagents by inversion or Vortex.

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. 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 washings.
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. 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 washings.
8. Add 100 µl of the **Substrate Solution** to each well.
9. 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.
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 %) :

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

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

IDvet Innovative Diagnostics

Certified management system

ID Screen®
Influenza N1 Antibody Competition

ELISA kit for the detection of specific antibodies against the avian Neuraminidase N1 of the Influenza A virus in bird sera

For *in vitro* use

May 2015:

Standardization of avian range testing procedures

- **Dilute samples and controls in Dilution buffer 14 (instead of Dilution buffer 8)**
- **Add 25µl of sample and controls (instead of 50µl previously)**
- **Incubate sera for 1 hour (instead of 1h30)**
- **Incubate Substrate solution for 15 min (instead of 10 min)**
- **Cut-off of 50-60% instead of 60%**

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