DATA SHEET

Influenza A H5N1 (Avian Flu) HA ELISA Kit

Catalog No. CSB-E17980

(96T)

- This immunoassay kit allows for the in vitro semi-quantitative determination of **influenza A H5N1 (avian flu) HA** concentrations in **birds Anus swabs, human Buccal Swab, human nasal swab and intact or lytic virus from chick embryo** .
- **Expiration date** **six** months from the date of manufacture
- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

PRINCIPLE OF THE ASSAY

The microtiter plate provided in this kit has been pre-coated with H5N1 (Avian Flu) HA antibody. Samples are then added to the appropriate microtiter plate wells with a HRP-conjugated antibody preparation specific for H5N1 (Avian Flu) HA and incubated. Then substrate solutions are added to each well. Only those wells that contain H5N1 (Avian Flu) HA and HRP-conjugated antibody preparation specific for H5N1 (Avian Flu) HA will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ±2 nm.

MATERIALS PROVIDED

Reagent	**Quantity**
Assay plate	1
virus lysis solution	5 x 20 ml
HRP-conjugate	1 x 12 ml
Wash Buffer	2 x 20 ml (20×concentrate)
Substrate A	1 x 6 ml
Substrate B	1 x 6 ml
Stop Solution	1 x 6 ml
Positive Control	1 x 1 ml
Negative Control	1 x 1 ml

STORAGE

1. Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag. The test kit may be used throughout the expiration date of the kit. Refer to the package label for the expiration date.
2. Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.
3. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

1. **Wash Buffer** If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to prepare 400 ml of Wash Buffer.

Precaution: The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.

SAMPLE PREPARTION

Solid samples (e.g. dejection):prepare with virus lysis solution(1:10).This can be achieved by adding 0.1g solid sample to 1ml of virus lysis solution. centrifuge and collect the supernates for assay.

Swab: add 1ml of virus lysis solution, centrifuge and collect the supernates for assay.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, and controls be assayed in duplicate.

1. Set three Negative Control wells, two Positive Control wells.Set one Blank well.

2. If the samples are in liquid and do not prepared by virus lysis solution, add 50ul virus lysis solution and 50ul samples. If the samples are already prepared by virus lysis solution, add 100ul samples. Add 100ul negative control and positive control, respectively.
3. Cover with the adhesive strip. Incubate for 60 minutes with an agitator with medium speed at room temperature.
4. Fill each well with Wash Buffer (about 200μl), stay for 10 seconds and Spinning. Repeat the process for a total of five washes. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100μl of **HRP-conjugate** to each well(Not to Blank well). Incubate for 30 minutes at 37℃. HRP-conjugate may appear cloudy. Warm up to room temperature and mix gently until solution appears uniform.
6. Wash plate **five** times as before.
7. Add 50μl of **Substrate A** and 50μl of **Substrate B** to each well. Incubate for 30 minutes at 37℃. Keeping the plate away from drafts and other temperature fluctuations in the dark.

8. Add 50µl of **Stop Solution** to each well when the first four wells containing the highest concentration of standards develop obvious blue color. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well within 10 minutes, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

For calculation the valence of influenza A H5N1 (avian flu) HA, compare the sample well with control..

Positive Control OD values must no less than 0.5.

Negative Control OD values must no more than 0.1.

- If the Negative Control OD values high than 0.1, discard it.
- If all the Negative Control OD values high than 0.1, repeat the test.
- If the ODnegative ＜0.03, calculate as 0.03.

A cutoff value was defined as the average Negative Control value plus 0.15.

While OD_{sample}≧cutoff value: Positive

While OD_{sample}＜cutoff value: Negative

LIMITATIONS OF THE PROCEDURE

- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- Centrifuge vials before opening to collect contents.
- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

- When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

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