# Avian Influenza H5 Virus Antibody (AIV-H5) ELISA Kit

Technical Manual

(ELISA)

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## Product Information

### Intended Use

Avian influenza, also known as European fowl plague, is an acute and highly lethal contagious disease affecting birds, caused by Influenza A virus. It is characterized by acute septicemic death or asymptomatic carrier status.

This assay is designed to detect Avian Influenza H5 Virus (AIV-H5) antibodies in the serum or plasma of avian species. It can be employed for the assessment of immunization efficacy and as a supportive diagnostic tool.

### Principle and Application

This kit comprises a coated Microtiter Plate with AIV-H5 antigen, antibody solution, HRP conjugate, and other accompanying reagents. It employs the principle of competitive enzyme-linked immunosorbent assay to detect antibodies against the AIV-H5 in the serum or plasma of avian species. During the experiment, diluted samples and antibody solution are added to the plate. If the sample contains AIV-H5 antibodies, they will compete with the antibody solution to bind with the antigen on the plate, thereby preventing subsequent color development. Conversely, color development occurs in the absence of such antibodies. The intensity of the color is inversely proportional to the specific antibody content in the sample. After adding stop solution to terminate the reaction, the product turns yellow. The absorbance values in each well are measured at a wavelength of 450nm using an microtiter plate reader, allowing determination of the presence of AIV-H5 antibodies in the sample.

### Composition of the Kit

| Reagent | Specification | | |
|---|---|---|---|
| Microtiter Plate | 96wells | 96wells×2 | 96wells×5 |
| HRP conjugate (red cap) | 1×11mL | 2×11mL | 2×26mL |
| Antibody Solution (blue cap) | 1×6mL | 1×11mL | 1×26mL |
| Concentrated Wash Buffer (20×) (white cap) | 1×40mL | 1×40mL | 1×200mL |
| Substrate Reagent A (white cap) | 1×6mL | 1×11mL | 1×26mL |
| Substrate Reagent B (black cap) | 1×6mL | 1×11mL (brown cap) | 1×26mL |
| Stop Solution (yellow cap) | 1×6mL | 1×11mL | 1×26mL |
| Positive Control (red cap) | 1×1.0mL | 1×1.5mL | 1×2.0mL |
| Negative Control (green cap) | 1×1.0mL | 1×1.5mL | 1×2.0mL |
| Adhesive Membrane | 1 | 2 | 5 |
| Sealed bag | 1 | 1 | 2 |
| Instructions | 1 | 1 | 1 |

### Storage conditions

The kit shall be stored at 2-8 °C. Avoid moisture.

Shelf life: 12 months. Please use within 2 months after opening. The date of manufacture is presented in the label of the box.

### Materials Required but Not Supplied

**Equipment:** microplate reader, adjustable micropipette, constant temperature device (37°C), centrifuge.

**Reagents:** 1mol/L hydrochloric acid (For yolk sample only.)

## Experimental preparation

***Restore all reagents and samples to room temperature (adjust to around 25°C) for more than 30 min before use. This is a crucial step to ensure there is no precipitation in the reagents.***

**1.Sample Preparation:**

Serum/plasma: It should be clear, without hemolysis or contamination. Samples can be stored at 2-8°C for up to 1 week, and for long-term storage, they should be kept at -20°C.

Yolk: Take 1mL of fresh yolk, add 7mL of physiological saline, shake and mix thoroughly. Then adjust the pH to 5.0 using 1mol/L hydrochloric acid. Centrifuge at 6000 rpm for 20 minutes. Collect the supernatant as the sample.

**2.Solution preparation:** Dilute the concentrated wash buffer (20×) by a factor of 20 (Concentrated wash buffer/Deionized water= 1: 19). What obtained is the working wash buffer.

Please note that the labware must be clean. Use disposable pipette tips to avoid contamination of interference results.

## ELISA procedure

Place all reagents and samples to room temperature (adjust to around 25°C) for 30min. Gently shake the reagent bottles before use.

Take out the frame of the microplate along with the

required number of wells. Then place the unused microplate wells into the sealed bag with the desiccant provided. Store the remaining kit in the refrigerator at 2-8°C.

1.Put the required number of the wells on the plate and set up 2 wells each for negative/positive control.

2.Add 50μL of **negative control** to each negative control well. Then add 50μL of **positive control** to each positive control well. For each sample well, first add 40 μL of **working wash buffer,** then add 10μL of the sample.

3.Add 50μl of **antibody solution** to each well, shake gently by hand (or use a microplate shaker) for 5s, cover with adhesive membrane and incubate at 37°C (water bath recommended) in the dark for 30 minutes.

4.Discard the liquid from the wells. Add 350μL of **working wash buffer** to each well, let stand for 30 seconds, then discard. Repeat the washing process 5 times. Invert the plate and tap it against a thick absorbent paper (or lint-free cloth), with a soft towel placed underneath. (Bubbles that are not removed after tapping dry can be punctured with a clean pipette tip).

5.Add 100μL of **HRP conjugate** to each well, cover with adhesive membrane, and incubate at 37°C in the dark for 30 minutes.

6.Washing. Same as step **4.**

7.First, add 50μL of **substrate reagent A** to each well, followed by 50μL of **substrate reagent B.** shake gently by hand (or use a microplate shaker) for 5s, cover with adhesive membrane, and incubate at 37°C in the dark for 15 minutes.

8.Add 50μL of **stop solution** to each well and shake gently by hand (or use a microplate shaker) for 5s. Read absorbance (**A value**) at 450nm with microplate reader (with 630nm as a reference wavelength). Finish this step within 10min.

**Reference Value**

Under normal experimental conditions, the A value of the negative control should be ≥1.0, and the A value of the positive control should be ≤ 50% of the A value of the negative control.

**Interpretation of Test Results**

1. PI(Percentage Inhibition%)=$(1 - \frac{A_S}{A_{NC}}) \times 100\%$ ,

If PI is ≥50%, it is considered positive; if PI is <50%, it is considered negative."

$A_S$—the A value of the sample;

$A_{NC}$—the average A value of negative controls.

**2. For unvaccinated chicken flocks:** A positive result indicates a possible infection, and it should be analyzed in conjunction with clinical data.

**3. For vaccinated chicken flocks:** Monitor and record the antibody levels of the samples. Analyze the distribution of antibody levels and the immune status of the flock based on the results.

**Limitations of the Test Method**

This test is only for the qualitative detection of AIV-H5 antibodies. Based on the PI Index, a rough assessment of the antibody level as strong, medium, or weak can be made.

## Attention

1. During the experiment, gloves and lab coats should be worn. Strict and comprehensive disinfection and isolation protocols should be followed. All experimental waste should be treated as infectious material.

2. The stop solution is corrosive. Avoid contact with skin and clothing. If accidentally contacted, rinse immediately with a large amount of tap water.

3. When taking the microtiter plate out of a refrigerated environment, it should be brought to room temperature before opening the bag. Unused microplate wells should be stored in the sealed bag with a desiccant.

4. During washing, each well should be filled completely with liquid to prevent any residual enzyme on the well's rim from remaining unwashed.

5. The samples used for testing should be kept fresh.

6. The determination of test results must be based on the readings from the microplate reader.

7. Components from different lot numbers must not be mixed.