

## **Avian Influenza Virus Antibodies ELISA Kit**

Catalog No: E-AD-E034

96T

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

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Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

## Test principle

This kit applies Competitive-ELISA as the method for the in vitro qualitative detection of concentration of antibodies to Avian Influenza Virus (AIV) in samples. The ELISA Microtiter plate provided in this kit has been pre-coated with the AIV antigen. In the experiment, add control serum, samples and Antibody Working Solution into plate. AIV antibodies in the samples will compete with the antibody in the working solution to bind with the antigen pre-coated on the Microplate. Then wash to remove unbound antibodies and other components, add the HRP Conjugate to specifically bind with the compound of antibody and antigen on the Microplate and the unbound HRP Conjugate will be removed by washing. Add TMB substrate to the wells, it will react with the enzyme and become blue, the shade of color is of negative correlation with AIV antibody levels in the samples. At last, end the reaction by adding Stop Solution to produce a yellow product. Measure the absorbance value of each well by using a Microplate Reader with 450 nm wavelength, then we can judge whether AIV antibody exist in the sample.

## Kit components

Item	Specifications
ELISA Microtiter plate	96 wells
Antibody Working Solution	5.5 mL
HRP Conjugate	11 mL
20×Concentrated Wash Buffer	40 mL
Substrate Reagent A	6 mL
Substrate Reagent B	6 mL
Stop Solution	6 mL
Positive Control	1 mL
Negative Control	1 mL
Plate Sealer	3 pieces
Sealed Bag	1 piece
Manual	1 copy

## Experimental instrument

Microplate Reader with 450nm wavelength filter or dual-wavelength (450/630nm)

High-precision transferpettor, EP tubes and disposable pipette tips

37°C incubator or water bath

Deionized or distilled water

Absorbent paper

## Reagent preparation

1. **Serum/plasma:** Use the conventional method to prepare serum/plasma, the serum/plasma must be clear, no hemolysis and no pollution. Samples can be conserved at 2~8 °C in 1 weeks, and it should be stored at - 20°C for a long term storage.  
**Yolk:** Take 2 mL of fresh yolk and add 2 mL of normal saline, oscillate to mix fully. Centrifuge at 3000 rpm for 15 min, take the supernatant for detection.
2. **Wash Buffer:** The 20×**Concentrated Wash Buffer** should be adjusted to room temperature before used, then dilute it with deionized or distilled water at 1:19.
3. Bring all reagents to room temperature (18~25°C) for 30 min before use.

## Assay procedure

1. **Number:** Take out the Micro-plate, set 2 wells for negative/positive control respectively. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2~8°C.
2. **Add sample:** Add 50 µL of positive/negative control to the wells respectively, add 10 µL of sample and 40 µL of **Wash Buffer** to each sample well.
3. **Incubate:** Then add 50 µL of **Antibody Working Solution** to each well. Gently tap the plate to ensure thorough mixing, incubate at 37°C for 30 min.
4. **Wash:** Aspirate each well and wash, repeat the process 5 times, immerse 30-60 sec each time. Wash by filling each well with Wash Buffer (approximately 300 µL) (a squirt bottle, multi-channel pipette, manifold dispenser or automated washer are needed). Complete removal of liquid at each step is essential. After the last wash, remove remained Wash Buffer by aspirating or decanting. Invert the plate and pat it against thick clean absorbent paper.
5. **HRP conjugate:** Add 100 µL of HRP Conjugate into each well, and incubate at 37°C for 30 min.
6. **Wash:** Repeat Step 4 for washing.
7. **Color Development:** Add 50 µL of Substrate Reagent A and 50 µL of Substrate Reagent B into each well, gently tap the plate to ensure thorough mixing, incubate for 15 min at 37°C. Protect from light.
8. **Stop reaction:** Add 50 µL of Stop Solution into each well, gently mix.
9. **OD Measurement:** Adjusted zero with the blank control, measure the absorbance value (A-value) of each well by using a Micro-plate Reader with 450 nm wavelength (use 630 nm as reference wavelength).

## Reference value

Normally, the OD of negative control  $\geq 1.0$  and the A-value of positive control  $\leq 50\%$   $\times$  A-value of negative control.

## Interpretation of the results

1.  $PI = (1 - \text{Sample OD} / \text{Average of negative control ODs}) \times 100\%$ .
2. Unimmunized chicken : Positive result :  $PI \geq 50\%$ ; Negative result :  $PI < 50\%$
3. Immunized chicken: The antibody levels at the time of the sample were monitored and recorded, and the distribution of antibody levels and the trend of immune status of the flock were analyzed based on the results.

**Note:** The negative result of this test suggests that the concentration of antibody for the tested samples is not enough, it is recommended that this animal should get immunized with corresponding vaccine.

## Limitations of this test method

This test is only used as the qualitative detection of AIV antibody in serum/plasma samples of Poultry. A rough estimate (high, general, low) of the antibody concentration can be calculated according to the PI values.

## Notes

1. Wear gloves and work clothes during experiment, and the disinfection and isolation system should be strictly executed. All the waste should be handled as contaminant.
2. The stop solution is corrosive, it should be avoided to contact with skin and clothing. Wash immediately with plenty of water if contact it carelessly.
3. The ELISA plate obtained from cold storage conditions should be adjusted to room temperature before use. The unused plate should be kept in a sealed bag with desiccant.
4. Concentrated Wash Buffer at low temperature condition is easy to crystallize, it should be adjusted to room temperature in order to dissolve completely before use.
5. Each well must be filled with liquid when washing in order to prevent residual free enzyme.
6. The tested sample should be kept fresh.
7. The results shall depend on the readings of the microplate Reader.
8. Do not use components from different batches of kit.

## Storage and Expiry date

Store at 2~8 °C with shading light for 12 months.

Please store the opened plate at 2~8°C, protect from light and moisture. The valid period is 2 months.

**Valid Period:** expiration date is on the packing box.