Avian Influenza Virus H7 Antibodies ELISA Kit

Catalog No: E-AD-E129

96T/96T*2/96T*5

Version Number:	V1.2
Replace version:	V1.1
Revision Date:	2025.05.12

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.

Phone: 240-252-7368(USA) Fax: 240-252-7376(USA)
Email: techsupport@elabscience.com
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Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Product introduction

This kit applies the Competitive-ELISA as the method for the in vitro qualitative detection of concentration of antibodies to Avian Influenza Virus H7 (AIV-H7) in samples. The ELISA Microtiter plate provided in this kit has been pre-coated with the AIV-H7 antigen. In the experiment, add control serum, samples and Antibody Working Solution into plate.AIV-H7 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-H7 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-H7 antibody exist in the sample.

Kit components

Item	Specifications
ELISA Microtiter plate	96 wells
Antibody Working Solution	6 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

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

Experimental instrument

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

High-precision transferpettor, EP tubes and disposable pipette tips

37℃ incubator or water bath

Deionized or distilled water

Absorbent paper

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. FOR RESEARCH USE ONLY. ELISA Microtiter plate should be covered by plate sealer. Avoid th e kit to strong light.
4. Concentrated Wash Solution 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. **Each reagent is optimized for use in the E-AD-E129. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-AD-E129 with different lot numbers.**
9. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.

Storage and expiry date

Store at 2-8℃. Avoid freeze.

Please store the opened plate at 2-8℃, the shelf life of the opened kit is up to 1 month.

Expiry date: expiration date is on the packing box.

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 ℃ in 1 weeks, and it should be stored at - 20℃ 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.

Assay procedure

Restore all reagents and samples to room temperature before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming.

1. **Number:** Take out the Microtiter 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℃. **Standard and Samples need test in duplicate.**
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℃ for 30 min.
4. **Wash:** Remove the liquid in each well. Immediately add 350 μL of **Wash Buffer** to each well and wash. Repeat wash procedure for 5 times, 30 s intervals/time. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
5. **HRP conjugate:** Add 100 μL of **HRP Conjugate** into each well, and incubate at 37℃ 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℃. Protect from light.
8. **Stop reaction:** Add 50 μL of **Stop Solution** into each well, gently mix.
9. **OD Measurement:** Measure the absorbance value (A-value) of each well by using a Microplate Reader with 450 nm wavelength (use 630 nm as reference wavelength).

Reference value

Normally, the OD of negative control ≥ 1.0 and the A-value of positive control ≤ 50% ×A-value of negative control.

Interpretation of the results

1. PI= (1-Sample OD/Average of negative control ODs) ×100%.
2. Positive result :PI ≥ 50%
3. Negative result : PI ＜ 50%.
4. Unimmunized animal: positive result indicates that it may be infected with AIV-H7.
5. Immunized poultry: 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

1. This test is only used as the qualitative detection of AIV-H7 antibody in serum and plasma samples of Poultry. A rough estimate (high, general, low) of the antibody concentration can be calculated according to the PI values.
2. The detection results of this kit are only for clinical reference. For confirmation of the diagnosis, please combine the clinical symptoms and other methods of detection, this detection cannot be used as the only criteria for clinical diagnosis.
3. All high sensitivity immune experiment system exists potential non-specificity. Therefore, unacceptable positive results may be caused by biological false positive of ELISA method.
4. In the early stage of infection, antibody did not occur or has a low titer, and these situations will lead to negative results.