Avian Influenza Virus H5 Antibodies ELISA Kit

Catalog No: E-AD-E045

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.

Phone: 240-252-7368(USA) Fax: 240-252-7376(USA)
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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 is comprised by HRP conjugate, other auxiliary reagents, ELISA Microtiter plate pre-coated with the Avian Influenza Virus H5 (AIV-H5) antigen. Apply the principle of enzyme-linked immunoassay (ELISA) to detect AIV-H5 antibody in serum of poultry animals (chicken, duck, goose, etc.). During the experiment, add control serum and samples into the ELISA Microtiter plate. If AIV-H5 antibodies exist in the samples, it will compete with the antibody in the antibody 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 microtiter plate. The unbound HRP conjugate will be removed by washing. Substrate Reagent is added into the well, it will react with the enzyme and become blue. The color shade is negative correlation with AIV-H5 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-H5 antibody exist in the sample.

Kit components

Item	Specifications
ELISA Microtiter plate	96 wells
HRP Conjugate	11 mL
10×Concentrated Wash Buffer	100 mL
Sample Diluent	30 mL
Substrate Reagent	11 mL
Stop Solution	15 mL
Positive Control	1 mL
Negative Control	2 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.

Other materials required but not supplied

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
Physiological saline solution

Notes

1. Wear gloves and work clothes during experiment, and the disinfection and isolation system should be strictly performed. 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 contacted 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 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 keep fresh.
7. The results shall depend on the readings of the Microplate Reader.
8. **Each reagent is optimized for use in the E-AD-E045. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-AD-E045 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:** Use the conventional method to prepare serum, the serum 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.
2. **Wash Buffer**: The **10×Concentrated Wash Buffer** should be adjusted to room temperature before use, then dilute it with distilled or deionized water at 1:9.

Assay procedure

Restore all reagents and samples to room temperature (25℃) before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2-8℃.

1. **Number:** number the sample and control in order (multiple well), and keep a record of control wells and sample wells. Set 2 wells for negative/positive control respectively.
2. **Add sample:** add 100μL of **positive/negative control** to positive/negative control well, add 40μL of **serum sample** and 60μL of **Sample Diluent** to each sample well, and mix thoroughly.
3. **Incubate:** Cover the plate sealer incubate at 37℃ for 30 min in shading light.
4. **Wash:** remove the liquid in each well. Immediately add 250 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, cover the plate sealer and incubate at 37℃ for 30 min in shading light.
6. **Wash:** repeat step 4 for washing.
7. **Color Development:** add 100 μL of **Substrate Reagent** into each well, Cover the plate sealer and mix thoroughly, incubate at 37℃ for 15 min in shading light.
8. **Stop reaction:** add 50 μL of **Stop Solution** into each well, mix thoroughly.
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:

Average $OD_{negative\ control}$ > 0.4

($OD_{positive\ control}$ / Average $OD_{negative\ control}$) <0.4.

Interpretation of the results

1. S/N value = OD_{Sample} / Average $OD_{negative\ control}$
2. Positive result : S/N value ≥ 0.5
3. Negative result : PI S/N value < 0.5
4. Unimmunized chicken: positive result indicates that it may be infected with AIV-H5
5. 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.

Limitations of this test method

1. The detection results of this kit are only for reference. For confirmation of the result, please combine the symptoms and other methods of detection, this detection cannot be used as the only criteria for result.
2. Because the H5, H7, and H9 subtypes of avian influenza viruses are highly similar in certain epitopes, these epitopes may be recognized by the same antibodies, resulting in cross-reactivity. This is a limitation of immunological based approaches.