Anatine Avian Influenza Virus Antibodies ELISA Kit

Catalog No: E-AD-E044

96T

Version Number:	V1.2
Replace version:	V1.1
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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.

Toll-free: 1-888-852-8623 Tel: 1-832-243-6086 Fax: 1-832-243-6017
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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 uses the blocking ELISA method for the detection of anatine avian influenza virus antibodies in the serum of anatine. anatine avian influenza antigen is pre-coated on the micro-wells of the microtiter plate strips. During the test, diluted test serum is added and incubated. If the sample contains anatine avian influenza antigen-specific antibodies, they will bind to the antigen coated on the plate. After washing to remove unbound antibodies and other components, enzyme-labeled anti-anatine avian influenza antibody is added. The antibodies in the sample block the binding of the enzyme-labeled antibody to the coated antigen. After washing to remove unbound enzyme conjugate, TMB substrate solution is added to the wells. The blue signal produced by enzymatic catalysis is inversely proportional to the antibody content in the sample. After adding stop solution to terminate the reaction, the absorbance (OD value) of the reaction wells is measured at 450 nm using a microplate reader.

Kit components

Item	Specifications
ELISA Microtiter plate	96 wells
HRP Conjugate	11 mL
10×Concentrated Wash Buffer	100 mL
Substrate Reagent	11 mL
Sample Diluent	30 mL
Stop Solution	15 mL
Negative Control	2 mL
Positive Control	1 mL
Plate Sealer	1 piece
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
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-E044. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-AD-E044 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.

Sample 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 to make the sediment dissolved fully before use, then dilute it with 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. **Samples need test in duplicate.**
2. **Add sample:** add 100 μL of **positive/negative control** to positive/negative control well, add 60 μL of **Sample Diluent** and 40 μL of **Serum** to each sample well.
3. **Incubate:** cover the plate sealer and mix thoroughly, incubate at 37℃ for 60 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, 30s 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.

Reference value

Normally, the A-value of negative control > 0.40, the A-value of positive control / A-value of negative control < 0.40.

Interpretation of the results

1. **S / N** = OD_{Sample} / Average $OD_{negative\ control}$.
2. Negative result : S / N ≥ 0.50;
3. Positive result : S / N < 0.50.

Limitations of this test method

1. This test is only used as the qualitative detection of AIV antibody in serum of anatine.
2. 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.