Avian Leukosis Complex Antigen ELISA Kit

Catalog No: E-AD-E074

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

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 is comprised by HRP conjugate, other reagents and ELISA Microtiter plate pre-coated with Avian Leukosis (ALV) P27 antibodies. Apply the principle of enzyme-linked immunoassay (ELISA) to detect ALV-Ag in cloacal orifice, yolk of poultry. During the experiment, add control and samples into the ELISA Microtiter plate, ALV-Ag will be bound with the antibodies on the ELISA Microtiter plate. Then wash the plate to remove unbound components, horseradish peroxidase (HRP) conjugate is added to each ELISA Microtiter plate well. The unbound HRP Conjugate will be removed by washing and substrate reagent is added for color development. At last, end the reaction by adding Stop Solution to produce a yellow product. There is a positive correlation between the OD value of samples and the concentration of ALV-Ag. Measure the absorbance value of each well by using a microplate reader with 450 nm (630 nm) wavelength, then we can judge whether ALV antibody exist in the sample.

Kit components

Item	Specification
ELISA Microtiter plate	96 wells
HRP Conjugate	25 mL
25×Concentrated Wash Buffer	25 mL
Substrate Reagent A	15 mL
Substrate Reagent B	15 mL
Stop Solution	15 mL
Positive Control	2 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.

Experimental instrument

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

High-precision transferpettor, EP tubes and disposable pipette tips

37℃ incubator or water bath

Deionized or distilled water

Absorbent paper

NaCl, KCl, Na_2HPO_4, KH_2PO_4

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-E074. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-AD-E074 with different lot numbers.**

Storage and expiry date

Store at 2-8℃. Avoid freeze.

Please store the opened plate at 2-8℃, protect from light and moisture.

Expiry date: expiration date is on the packing box.

Sample preparation

1. **Solution preparation**

   Please prepare solution according to the number of samples. Don't use up all components at once!

   Wash Buffer: Adjust the **25×Concentrated Wash Buffer** to room temperature before use, shake it and make it dissolve fully if appearing salt crystals, then dilute it with deionized water at 1:24 (eg: 1 mL 25×Concentrated Wash Buffer +24 mL deionized water)

   0.01M PBS Solution (pH=7.4)： Dissolve 8.0 g of NaCl, 0.2 g of KCl, 1.14 g of Na_2HPO_4, 0.24 g of KH_2PO_4 to 1000 mL with deionized water. It can be stored at room temperature for a long time.

2. **Sample preparation**

   Cloacal orifice:

a) The cotton swab should be inserted into the **Cloacal orifice** and rotated repeatedly on the inner wall (sample collection affects the detection result, ensure it adequately).

b) Immediately, insert the cotton swab into1 mL of 0.01 M PBS (pH=7.4) tube containing diluent, stir the swab until the sample is dissolved into the diluent fully. Discard the cotton swab after wiping it against the wall of the tube. The sample tube is frozen once in the refrigerator below -20 ℃, then restore sample tube to room temperature.

c) Take 100 μL of sample solution for analysis.

   Yolk: Take 100 μL of the yolk for analysis.

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 100 μL of sample to the sample wells.
3. **Incubate:** cover the plate sealer and mix thoroughly, incubate at 37℃ for 30 min in shading light.
4. **Wash:** remove the liquid in each well. Immediately add 260 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 50 μL of **Substrate Reagent A** and 50 μL of **Substrate Reagent B** into each well and mix thoroughly. Cover the plate sealer and mix thoroughly, incubate at 37℃ for 10 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). **Note: Read the results within 5 min**

Reference value

Normally, average absorbance of negative control < 0.5 and Average absorbance of positive control ≥ 1.0.

Analysis of results

1.

$$S/P = \frac{\text{Absorbance of sample- Average absorbance of negative control}}{\text{Average absorbance of positive control- Average absorbance of negative control}}$$

2. Positive result: $S/P \geqslant 0.2$
3. Negative result: $S/P < 0.2$

Limitations of this test method

1. This test is only used for the qualitative detection of ALV-P27-Ag in samples of poultry.
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.