

Bird Flu (H9N1) antibody ELISA test kit

**INSTRUCTION MANUAL
FOR PRODUCT No: LT81003AYSL**



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MANUAL VERSION 1.01

SUMMARY

Bird flu (Avian influenza, AI) is an acute contact venereal toxicity infectious disease against the domestic poultry industry at present. Vaccination is the most effective way to prevent and control it, the antibody level after immune reflects the vaccine effect, which directly related to immune resistance to avian influenza virus in chicken flocks. This product is applicable to the different species, different age in chicken serum specific antibody detection of H9N1. It can be used for H9N1 virus vaccine immune time of analysis, evaluation of immune effect, chickens with H9N1 in immune status.

PRINCIPLE

The kit is based on an indirect enzymatic immunoassay (Indirect ELISA). The antigen is coated on plates. When a sample serum contains specific antibodies against virus, they will bind to the antigen on plates. Wash the unbound antibodies and other components. Then add a specific Enzyme conjugate. After incubation and washing, add the TMB substrate. A colorimetric reaction will appear, measured by a spectrophotometer (450 nm).

MATERIALS SUPPLIED WITH THIS KIT

1	H9N1 Antigen coated plate	96T X 1	7	Stop reaction solution	6 ml×1
2	Enzyme conjugate	11 ml×1	8	Positive control	1ml
3	20X concentrated Washing solution	40ml×1	9	Negative control	1 ml
4	Substrate A	6 ml×1	10	Adhesive Foil	2 pieces
5	Substrate B	6ml×1	11	Instruction	1 piece
6	Sample diluent	50 ml×1	12	Sealed bag	1 piece

MATERIALS REQUIRED BUT NOT PROVIDED

- 1) Microplate Reader (wave length: 450/630 nm).
- 2) 37 °C incubator.
- 3) Micropipettes, adjustable.

SAMPLE PREPARATION

1. Take animal whole blood, get serum by regular method, the serum need to be clear, no hemolysis, no pollution. For short-term storage, the sample can store at 2~8 °C, for long-term storage, at -20°C.
2. Dilute serum with Sample diluent at 100 times (such as add 2µl serum into 198µl sample diluent, stir evenly). Do not dilute Positive control and Negative control.
3. Return the 20x Concentrated washing solution into room temperature (about 25°C) before use, shake to dissolve the precipitated salt, then dilute it with distilled water or deionized water at 20 times.

TEST PROCEDURE

1. Add 100µl sample diluent into blank control well. Add Negative control to negative control well, 100µl/well, Positive control to positive control well, 100µl/well; for sample well, add diluted serum 100µl/well.
2. Mix evenly, incubate at 37 °C for 30 minutes.
3. Discard liquid of the wells and fill all wells with washing solution, incubate for 30s and discard. Repeat washing procedure 5 times as above, pat to dry.
4. Add enzyme conjugate to each well, 100µl/well.
5. Incubate at 37 °C for 30 minutes.
6. Wash as step 3.
7. Add Substrate A 50µl/well, then Substrate B 50µl/well, mix evenly, incubation at 37 °C in dark for 10 minutes.
8. Add stop solution 50µl/well, mix evenly, use ELISA Reader to measure A value at 450nm (630nm as reference) of each well.

RESULTS

OD Value of negative control well should be ≤ 0.20 (Invalid if > 0.20);

OD Value of positive control well ≥ 0.60 (Invalid if < 0.60).

Calculation of C.O Value: $C.O = 0.13 + \text{Mean of Negative control well}$ (Calculate as 0.07 when the mean of Negative control well is lower than 0.07).

LIMITATION

The kit can only detect H9N1 IgG antibody in chicken serum or plasma qualitatively. Make crude evaluation strong, medium and weak of antibody level based on A value.

NOTES

- 1) Wear gloves and work clothes when operate, strictly sound and perform disinfection and isolation system.
- 2) The stop solution is corrosive, avoid touch skin and clothes, wash with tap water if touched.
- 3) Microplate removed from the refrigerated environment should be balancing to dry at room temperature, and seal the unused microplate with desiccant.
- 4) Wash solution is easily crystallized at low temperature, to be returned to room temperature when used to dissolve.
- 5) Add Washing solution to each well fully, to prevent orifice free enzyme, which cannot be washed
- 6) The test sample should be fresh.
- 7) Determination of the test results must be based on ELISA reader.
- 8) Never mix reagents from different batches.

Specifications: 96 wells/kit.

Expiry date: 12 months.

Storage: Storing at 2-8°C, in the dark.