Science for Society

Avian Influenza Virus A (AIV) antibody ELISA test kit

INSTRUCTION MANUAL

FOR PRODUCT No: LT82003AYSL2

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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.

In Breeders' immunization work, the most noteworthy is mass immunization vaccination timing issues. In the time between the two rounds of immunization, antibody levels of breeders gradually decreased over time, and at what level to immunization is a headache problem. As at high antibody levels to be immune, it is not only a waste of vaccines, increased economic costs, and high levels of antibodies and vaccines, affect the immune effects of vaccines, lead to immune failure; but immune at the lower levels of antibodies, antibody protection vacuum occurs, threaten the health of breeders.

This product is applicable to the different species, different age in chicken serum specific antibody detection of avian influenza virus. It can be used for avian influenza (virus) vaccine immune time of analysis, evaluation of immune effect, chickens with avian influenza in immune status.

PRINCIPLE

This kit is composed by AIV antigen coated microplate, Enzyme conjugate etc, to detect AI virus IgG antibodies in chicken serum or plasma by principle of enzyme immunoassay indirect method (ELISA).

MATERIALS SUPPLIED WITH THIS KIT

1	AIV Antigen coated microplate	96T X 2	7	Stop solution	7 ml×2
2	Enzyme conjugate	11 ml×2	8	Positive control	0.5 ml×2
3	20X concentrated Washing buffer	40ml×2	9	Negative control	0.5 ml×2
4	Substrate A	7 ml×2	10	Adhesive Foil	2 piece
5	Substrate B	7 ml×2	11	Instruction	1 piece
6	Sample diluent	50 ml×2	12	Sealed bag	2 piece

MATERIALS REQUIRED BUT NOT PROVIDED

1) Microplate Reader (wave length: 450/630 nm).

2) 37 ℃ incubator.

3) Micropipettes, adjustable.

SAMPLE PREPARATION

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℃, for long-term storage, at -20℃.

SAMPLE DILUTION

Dilute serum with Sample diluent at 100 times (such as add 5μl serum into 495μl sample diluent, stir evenly). Positive control serum and Negative control serum do not need dilute.

WASHING SOLUTION PREPARATION

1) Return the 20x Concentrated washing buffer into room temperature (about 25 ℃) before use, shake to dissolve the precipitated salt, then dilute it with distilled water or deionized water at 20 times.

TEST PROCEDURE

1) Return the kit to room temperature for 30mins before use.

2) Take the needed quantity microplate well, set 2 wells for negative control, 2 wells for positive control, seal the unused plate, store at 2-8℃ soon.

3) Add Negative control to negative control well, 50µl/well, Positive control to positive control well, 50µl/well; for sample well, add diluted sample 50ul/well;

4) Add Antibody working solution to each well, 50µl/well. Mix evenly, Incubation at 37 ℃ for 30 minutes.

5) 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.

6) Add enzyme conjugate to each well, 100µl/well. Incubation at 37 ℃ for 30 minutes.

7) Wash as step 5.

8) Add Substrate A: 50ul/well, then Substrate B: 50µl/well to each well, mix evenly, incubation at 37 ℃ in dark for 15 minutes.

9) Add stop solution: 50µl/well to each well, mix evenly, use ELISA Reader to measure A value at 450nm (630nm as reference) of each well.

RESULTS

1) Negative control well: In normal, A value of negative control well ≤0.1;

2) Positive control well: In normal, A value of positive control well ≥0.6;

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

RESULT JUDGE

When Sample A_{450} ≥C.O, the result is Positive;

When A450≤C.O, the result is negative.

LIMITATION

The kit can only detect AIV 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. Various experimental wastes should be treated as contaminants

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 balance to dry at room temperature, and seal the unused microplate with desiccant.

4) Wash solution is easily crystallized at low temperature, return to room temperature when used to dissolve.

5) Add Washing buffer 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 use reagents from different batches.

Specifications: 96 × 2 wells/kit.

Expiry date: 12 months.

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

Store the microplate at 2-8℃, to avoid moisture, for 2 months after open the package.