IMMUNOMART

VERSION 1.01

AVIAN INFLUENZA VIRUS ANTIBODY ELISA KIT

MANUAL

ELISAKITS.ONLINE

By Immunomart

IMMUNOMART

Avian Influenza Virus Antibody ELISA Kit

Catalogue Number.

Brief

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).

Specifications: 96 wells × 2.

Components

	Code item	**Spec.**
1	AIV antigen coated microplate	96T×1
2	Enzyme conjugate	11ml×1
3	Sample diluent	50ml×1
4	Substrate A	7 ml×1
5	Substrate B	7 ml×1
6	20x Concentrated washing solution	40 ml×1
7	Positive control serum	0.5 ml×1
8	Negative control serum	0.5 ml×1
9	Stop solution	7 ml×1
10	Adhesive Foil	1 piece

Material required not provided

1. Micropipettes: 0.5μL~10μL、10μL~100μL、100μL~1000μL
2. Disposable pipette tips
3. Measuring cylinder: 500 ml
4. 96 wells microplate reader
5. Distilled water or deionized water
6. Bottle or microplate washing machine

Sample preparation

Take animal whole blood, get serum by using regular method, the serum should bright and no hemolysis

Washing buffer preparation

Return 10X Concentrated washing buffer into room temperature before use, if there is salt crystals, shake to make it dissolved, then dilute it at 10 times with distilled water or deionized water. The diluted washing buffer can store at 4℃ for about 1 week.

Sample dilution

At serum dilution plate, dilute serum at 1:100 with sample diluent.
Notice: Negative control and Positive control do not need dilute. Exchange tip after taking sample every time, record the situation of the sample on plate accurately. Shake the sample evenly before adding it

Operation procedures

1 .Adding sample: Take out the required coated plates according to sample quantity (Can be detached) and record the sample position on a worksheet. For single-wave length test, set one blank control well, add nothing; for double-wave length test, do not set blank control well. Set 2 wells for negative control serum and 2 wells for positive control serum, add undiluted negative and positive control serum to its well accordingly, 100 μL/well. Others are wells for samples, add 100μL/well of Sample diluent solution, then add 1 μL serum sample separately (there will be color change after adding sample).
2. Mix gently for 30s, incubate at 37℃ for 30 min.
3. Remove adhesive foil. Pour the liquid out of the wells, add Washing solution (dillute the Wash Concentrate at 20 times with deionized water) into each well fully, stand for 1 min. Repeat 5 times, at last time pat to dry on absorbent paper.
4. Add 100 μL enzyme comjugate into each well (except the blank well, also do not add any liquid to blank well).
5. Cover plate with new adhesive foil. Incubate at 37 ℃ for 30 min.
6. Repeat step 3(washing).
7. Add 50μL substrate A and substrate 50μL B into each well, mix properly, incubate for 15 min at 37 ℃ in the dark with new adhesive foil.
8. Add 50μL stop solution into each well, mix gently and determine the result within 5-30 min.

9. For single-wavelength test, measure the A value with a photometer at 450 nm, set zero for the blank well, and read A value of each well; For double-wavelength test, measure the A value with a photometer at 450 nm (Reference-wavelength: 630 nm), read A value 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)*

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.
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, to be returned to room temperature when used to dissolve.
5) Add Washing solution to each well fully, to prevent orifice free enzyme, which can not 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.

storage: store at 2-8℃, dark, sealed, dry place, no frozen.
Expiry date: 12 months; date of production is on box.