VERSION 1.01

BIRD FLU SUBTYPE H9 ANTIBODY ELISA KIT

MANUAL

ELISAKITS.ONLINE

By Immunomart

Bird Flu Subtype H9 Antibody ELISA Test kit

Catalogue Number. IP100192

Product Usage

This kit is used to detect Bird Flu subtype H9 antibody in chicken serum, to assess antibody condition by Bird Flu subtype H9 vaccine in chicken farm and assist diagnosis of serological infected chicken.

Principle

The Bird Flu subtype H9 antibody ELISA 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).

Specifications: 96 wells × 2.

Components

	Code item	**Spec.**
1	H9 antigen coated microplate	1 plate of 96 wells
2	Enzyme conjugate	11 ml
3	10×concentrated washing buffer	100ml
4	Substrate solution	11 ml
5	Sample dilution	100ml
6	Stopping solution	11ml
7	Negative control	0.8ml
8	Positive control	0.8ml
9	Serum dilution plate	1 piece
10	Adhesive film	2 pieces
11	Instruction	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

Notes

1) All reagents should be adjusted to the room temperature and shake evenly before using, store at 2-8 ℃ after using
2) Do not exchange the reagents from the kits of different lot numbers to use. Avoid reagent pollution when using.
3) Substrate and stop solution may have excitant to skin and eyes, pay attention when using.
4) Do not expose TMB (Substrate B) to light and avoid it contact with antioxidants.
5) The wells should avoid damp or touching water after unsealing (Put the un-using microplate back to bag with dehydrator in 2~8 ℃ soon)
6) Deal all waste reasonable before dumping to avoid pollution.
7) Strictly adhere to instruction to get best result. All procedure including pipetting, timing and washing etc. must be accurate.
8) Serum dilution plate is disposable, do not use for second time; the MAX volume of it is 300μL/well.

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

Read the OD value with ELISA reader at 450nm(630nm as reference)
For the assay to be valid:
OD Value of Negative control (N)< 0.2, meanwhile average OD value of Positive control (P)>0.4.
Calculation method:
Sample OD value / Positive control OD Average value= S/P value
The result is judged by S/P value:
If S/P≥0.25, it is positive; If S/P< 0.25, it is negative.

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