

**BIRD FLU H5 SUBTYPE ANTIBODY ELISA
KIT
MANUAL**

Bird Flu H5 Subtype Antibody ELISA Test kit

Catalogue Number. IP100185

Brief

This kit is used to detect Bird Flu H5 subtype antibody in serum of poultry, wild birds etc., to assess antibody condition by Bird Flu H5 vaccine poultry and assist diagnosis of serological infected poultry.

This kit use blocking ELISA method, H5 antigen is pre-coated on enzyme micro-well strips, indirect detection of anti-H5 antibodies in serum by anti-monoclonal antibodies compete with antibodies in serum for binding sites in solid-phase vectors. When testing, add diluted serum sample, after incubation, if there is H5 virus specific antibody, it will combine with the pre-coated antigen, discard the uncombined antibody and other components with washing; then add enzyme labeled anti-H5 virus monoclonal antibody, antibody in sample block the combination of monoclonal antibody and pre-coated antigen; discard the uncombined enzyme conjugate with washing; Add TMB substrate in micro-wells, the blue signal by Enzyme catalysis is in inverse proportion of antibody content in sample, use ELISA reader at 450nm wavelenth to measure the absorbance A value in reaction wells after adding stop solution to stop the reaction.

Specifications: 96 wells × 2.

Components

	Code item	Spec.
1	H5 Ag Coated plates 96 wells	1 plate
2	Enzyme Conjugate	11 ml
3	10XConcentrated Washing buffer	100 ml
4	Substrate	11 ml
5	Sample dilution	100 ml
6	Stop solution	15 ml
7	Negative control	2 ml
8	Positive control	1 ml
9	Adhesive Foil	2 pieces
10	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°C 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. 630 nm), read A value of Take the antigen coated plate(the plate can be open and used for several times according to sample quantity each time), for every test, set 1 well for positive control and 2 wells for negative control, positive control and negative control do not need dilute, take 100ul directly and add into its well;
2. Add Sample dilution to reaction wells, 50ul/well, then add serum sample to the reaction wells, 50ul/well, blow and mix evenly(Do not mix use tips)
3. Cover it with Adhesive Foil, incubate at 37°C for 120minutes;
4. Open the adhesive foil, discard the liquid of the well, add diluted washing buffer to each well, 250ul/well, then discard the liquid, repeat the above step for 6 times, at last flap to dry with the absorbent paper;
5. Adding Enzyme Conjugate 100ul/well, Cover it with Adhesive Foil, incubate at 37°C for 30 minutes;
6. Open the adhesive foil, discard the liquid of the well, washing for 6 times as step 4), remember at last flap to dry with the absorbent paper;
7. Add substrate, 100ul/well, mix it evenly then cover it with Adhesive Foil, incubate at 37°C in dark for 15 minutes;
8. Add stop solution 50ul/well to stop the reaction, measure the result in 10 minutes

Results

Read the OD value with ELISA reader at 450nm(630nm as reference)

For the assay to be valid:

S/N \geq 0.55, Negative;

S/N $<$ 0.55, Positive

Notes

- 1) Return all reagents into room temperature before use, put the reagents at room temperature for at least 1 hour. Shake it evenly before use, and store back to 2-8°C after usage.
- 2) Do not mix use reagents from different kits and different lot no., prevent the reagents been polluted when using.
- 3) Substrate and stop solution may have irritation to skin and eyes, be careful to use.
- 4) Do not expose Substrate to strong light and avoid contact with the oxidant.
- 5) H5 Ag coated plates should be sealed and moisture-proof. Put back unused Micro-Well plate into dry foil bag and sealed at 2-8 °C.
- 6) All wastes should be treated well to avoid pollution before discarding.
- 7) Strict compliance with the operating instructions can get the best results. Pipetting operation, timing, and washing of the whole process must be precise.
- 8) H5 Ag Coated plates is disposable, do not repeat use.

storage: store at 2-8°C, dark, sealed, dry place, no frozen.

Expiry date: 12 months; date of production is on box.