VERSION 1.01

AVIAN EDS76 ANTIBODY ELISA KIT

MANUAL

ELISAKITS.ONLINE

By Immunomart

Avian EDS76 antibody ELISA kit

Catalogue Number. IP100189

Product Usage

This kit is used to detect Avian EDS76 virus antibody in chicken serum, to assess antibody condition by Avian EDS76 vaccine in chicken farm and assist diagnosis of serological infected chicken.

Principle

The Avian EDS76 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	EDS76 antigen coated microplate	1/2 plates96B wells
2	Enzyme Conjugate	12 ml
3	10X Concentrated washing buffer	50 ml
4	SubstrateA	6ml
5	Sample diluent	50 ml
6	Stop solution	6ml
7	Negative control	800 ul
8	Positive control	800ul
9	Serum dilution plate	1pieces
10	Adhesive Foil	2pieces
11	Instruction sheet	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 diluent plate is disposable, do not use for second time; the MAX volume of it is 300μL/well.

Operation procedures

Take pre-coated microplate (Can unseal for several time use as per sample quantity), add 100μL diluted serum to a well, meanwhile set 1 wells for Negative control serum, Positive control serum and blank control wells separately. Add 100 μL Negative/Positive control serum to its wells, only add 100μL sample diluent buffer in the blank control wells. Shake softly, incubate at 37℃ for 30 min.
2) Pour the liquid out of the wells, add 350 μL diluted washing solution to each well, static for 1 min, pour out. Repeat 3 times, then pat to dry on absorbent paper.
3) Add 100 μL Enzyme conjugate (except blank wells) to each well, and incubate at 37℃ for 30 min.
4) Repeat the step 2(washing). Remember pat to dry on absorbent paper at last.
5) Add 50 μL substrate A, then substrate B (50 μL) to each well, mix properly, react for 10 min at dark at 37℃ in dark.
6) Add stop solution one drop (50 μL) in each well, and measure the result within 10 min.

Results

Set zero for the blank well, and test the OD450nm (recommend 450nm and 630 nm) value on the microplate-reader. The conditions for the test to be tenable are that the positive control wells' average OD450nm value is greater than or equal to 0.6, and the negative control wells' average OD450nm value is less than 0.15.(all value should minus the average value of blank control)
If the sample's A450 value is greater than 0.2+ absorbance of negative control, it is judged to be positive; and if less than 0.2+ absorbance of negative control, negative. If absorbance of negative control is less than 0.05, calculate as 0.05

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