

AVIAN INFLUENZA (H5N1) ANTIBODY ELISA KIT

**INSTRUCTION MANUAL
FOR PRODUCT No: LT11003AYSL**



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MANUAL VERSION 1.01

1. Summary

This kit uses AIV H5N1 solid phase antigen to detect specific antibody against AIV H5N1 antigen, and has high sensitivity, specificity, reproducibility, and is easy to operate quickly and can be used in multiple times.

2. Reagents and contents

1 H5N1 Antigen coated Plate	96T×1
2 Enzyme conjugate H5N1-Ab (No.1 solution)	6 ml×1
3 Washing solution (No.2 solution)	50ml×1
4 Substrate A (No.3 solution)	6 ml×1
5 Substrate B (No.4 solution)	6 ml×1
6 Sample diluent (No.5 solution)	50 ml×1
7 Stop reaction solution (No.6 solution)	6 ml×1
8 Positive control H5N1-Ab	0.8 ml×1
9 Negative control H5N1-Ab	0.8 ml×1
10 Adhesive Foil	2 pieces
11 Sealed bag	1 piece
12 Instruction	1 piece

3. Materials Required But Not Provided

1 Microplate Reader (Including Wavelength 450nm/630 nm).

2 37°C Constant temperature box

3 Micropipettes, adjustable.

4. Sample requirement

1 The samples are serum, storage time should be for 7 days at 2-8 °C, if for long term, it should be kept at -20°C or below it. Avoid repeated freezing and thawing.

2 Avoid to use the samples containing sodium azide, high cholesterol, with bacteria and severe hemolytic

5. Test procedure

1. Washing solution preparation

Dilute washing solution (No.2 solution) with distilled water or deionized water at 1:10, e.g. add 900ml distilled water or deionized water in 100 ml washing solution, and mix thoroughly.

2. Sample diluent:

Dilute the serum sample with sample diluent (No.5 solution) at 1:100, e.g. 5 µl serum + 495 µl sample diluent, mix thoroughly.

3. Adding samples and controls

For each test, set 2 wells for Negative control, 2 wells for positive control. Add negative control, positive control respectively, 100µl/well. Set one blank control well, add nothing. For sample well, add diluted serum sample, 100µl/well. **Incubation at 37 °C in dark for 30 minutes**. Discard liquid of the wells and fill all wells with diluted washing solution, incubate for 1 minute and discard. Repeat washing procedure 3 times as above. At the

end of the washing step, carefully remove remaining fluid by tapping the strips on the tissue paper prior to the next step.

4. Adding Enzyme conjugate

Apart from the blank control well, add 100ul Enzyme conjugate (No.1 solution) into all wells and **incubate at 37 °C in dark for 30 minutes**. Discard liquid of the wells and wash 3 times as described in step 3.

5. Adding substrates

Mix Substrate A (No.3 solution) and Substrate B (No.4 solution) in equal volumes, add into each well, 100µl/well, **incubate at 37 °C dark for 10 minutes**.

6. Add one drop of Stop reaction solution (No.6 solution) into all wells. Before reading results with ELISA microtiter plate reader, set the reagent blank well A1 to zero . Measure the OD Value of all wells at 450 nm (reference wavelength: 630 nm).

6. Reference

Validation judge

OD Value of negative control well ≤ 0.15 (Invalid if > 0.15);

OD Value of positive control well ≥ 0.30 (Invalid if < 0.30).

7. Interpretation of results

OD value of sample \geq OD Value of Positive control mean X 1.1: H5N1 Antibody Positive;

OD value of sample $<$ OD Value of Positive control mean X 0.9: H5N1 Antibody Negative;

OD Value of Positive control mean X 0.9 $<$ OD value of sample $<$ OD Value of Positive control mean X 1.1: Skeptical, this sample need review;

OD Value of sample review \leq OD Value of Positive control mean X 1.1: H5N1 Antibody Negative

8. Limitation

This kit is only suitable for testing chicken serum.

9. Notes

- 1) Microwell should be sealed and moisture-proof. MicroWell plate removed from the refrigerated environment should be balanced moisture to dry at room temperature, then can be opened. Put back unused MicroWell plate into dry foil bag and sealed at 4 °C.
- 2) Before dropping reagent, shake dropping bottle gently to even, discard the first drop; Keep the dropping bottle upright when adding reagents.
- 3) Cover the gap tightly after using every time. Do not mix using the cap. Do not use reagents from different lot.
- 4) Treat the used kits and reagents as pollution material. The stop solution is corrosive, be careful to use.

10. Storage and Expiry date:

Store at 2-8°C in dark, no freeze. Shelf life: 12 months