



User's Manual

Avian Influenza Virus H9 Antibodies ELISA Kit



DEIA-NS2312-3



96T



This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

Assay kit to test avian Influenza virus H9 antibodies.

Principles of Testing

This kit is comprised by HRP conjugate, other reagents, ELISA Microtiter plate pre-coated with the Avian Influenza Virus H9 (AIV-H9) antigen. Apply the principle of enzyme-linked immunoassay (ELISA) to detect AIV-H9-Ab in serum, plasma and yolk of poultry animals. During the experiment, add control, samples, and antibody working solution into the ELISA microtiter plate, AIV-H9-Ab will compete with the antibody working solution to bind with the antigen pre-coated on the ELISA Microtiter plate. Then horseradish peroxidase (HRP) conjugate is added to each ELISA microtiter plate well, and substrate reagent is added for color development. There is a negative correlation between the OD value of samples and the concentration of AIV-H9-Ab. Measure the absorbance value of each well by using a microplate reader with 450 nm (630 nm) wavelength, then we can judge whether AIV-H9 antibody exist in the sample.

Reagents And Materials Provided

1. ELISA Microtiter plate, 96 wells
2. Antibody Working Solution, 6 mL
3. HRP Conjugate, 11 mL
4. 20xConcentrated Wash Buffer, 40 mL
5. Substrate Reagent A, 6 mL
6. Substrate Reagent B, 6 mL
7. Stop Solution, 6 mL
8. Positive Control, 1 mL
9. Negative Control, 1 mL
10. Plate Sealer, 3 pieces
11. Sealed Bag, 1 piece
12. Manual, 1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

Materials Required But Not Supplied

Microplate Reader with 450nm wavelength filter or dual-wavelength (450/630nm)

High-precision transferpette, EP tubes and disposable pipette tips

37° C incubator or water bath

Deionized or distilled water

Absorbent paper

Physiological saline solution (0.9%)

Storage

Store at 2-8°C. Avoid freeze.

Please store the opened plate at 2-8°C, the shelf life of the opened kit is up to 1 month.

Expiry date: expiration date is on the packing box.

Specimen Collection And Preparation

Serum/plasma: Use the conventional method to prepare serum or plasma, the serum or plasma must be clear, no hemolysis and no pollution. Samples can be conserved at 2-8°C in 1 weeks, and it should be stored at -20°C for a long term storage.

Yolk: Take 2 mL of fresh yolk and add 2 mL of physiological saline solution, oscillate to mix fully. Centrifuge at 3000 r/min for 15 min, take the supernatant for analysis.

Reagent Preparation

Wash Buffer: The 20xConcentrated Wash Buffer should be adjusted to room temperature before used, then dilute it with deionized or distilled water at 1:19.

Assay Procedure

Restore all reagents and samples to room temperature (25°C) before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2-8°C.

1. **Number:** number the sample and control in order (multiple well), and keep a record of control wells and sample wells. Set 2 wells for negative/positive control respectively. Samples need test in duplicate.
2. **Add sample:** add 50 µL of positive/negative control to positive/negative control well, add 10 µL of sample and 40 µL of Wash Buffer to each sample well.
3. **Incubate:** add 50 µL of Antibody Working Solution to each well. Cover the plate sealer and mix thoroughly, incubate at 37°C for 30 min in shading light.
4. **Wash:** remove the liquid in each well. Immediately add 300 µL of Wash Buffer to each well and wash. Repeat wash procedure for 5 times, 30 s intervals/time. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
5. **HRP Conjugate:** add 100 µL of HRP Conjugate into each well, cover the plate sealer and incubate at 37°C for 30 min in shading light.
6. **Wash:** repeat step 4 for washing.
7. **Color Development:** add 50 µL of Substrate Reagent A and 50 µL of Substrate Reagent B into each well, Cover the plate sealer and mix thoroughly, incubate at 37°C for 15 min in shading light.
8. **Stop reaction:** add 50 µL of Stop Solution into each well, mix thoroughly.
9. **OD Measurement:** Measure the absorbance value (A-value) of each well by using a Microplate Reader with 450 nm wavelength (use 630 nm as reference wavelength).

Interpretation Of Results

1. PI (Percentage of inhibition) = $(1 - \text{OD Sample} / \text{Average OD negative control}) \times 100\%$.
2. Positive result : PI $\geq 50\%$
3. Negative result : PI $< 50\%$.
4. Unimmunized animal positive result indicates that it may be infected with AIV- H9
5. Immunized animal: The antibody levels at the time of the sample were monitored and recorded, and the distribution of antibody levels and the trend of immune status of the flock were analyzed based on the results.

Reference Values

Normally, the OD of negative control ≥ 1.0 and the OD of positive control $\leq 50\% \times \text{OD of negative control}$.

Sensitivity

sensitivity $> 98\%$, specificity $> 98\%$

Precautions

1. Wear gloves and work clothes during experiment, and the disinfection and isolation system should be strictly executed. All the waste should be handled as contaminant.
2. The Stop Solution is corrosive, it should be avoided to contact with skin and clothing. Wash immediately with plenty of water if contact it carelessly.
3. FOR RESEARCH USE ONLY. ELISA Microtiter plate should be covered by plate sealer. Avoid the kit to strong light.
4. Concentrated Wash Solution at low temperature condition is easy to crystallize, it should be adjusted to room temperature in order to dissolve completely before use.
5. Each well must be filled with liquid when washing in order to prevent residual free enzyme.
6. The tested sample should be kept fresh.
7. The results shall depend on the readings of the Microplate Reader.
8. Each reagent is optimized for use in the CD kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other kits with different lot numbers.
9. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.

Limitations

1. This test is only used as the qualitative detection of AIV- H9 antibody in serum, plasma and yolk of poultry. A rough estimate (high, general, low) of the antibody concentration can be calculated according to the PI values.

2. The detection results of this kit are only for reference. For confirmation of the result, please combine the symptoms and other methods of detection, this detection cannot be used as the only criteria for result.