Avian Influenza Virus H9 subtype antibody ELISA Kit

AIV H9 Ab Test

Product Number: 0964-E20198-1
Product Unit: 1 plate, 96T

1. Introduction

Influenza, commonly known as "the flu", is an infectious disease caused by an influenza virus. Symptoms can be mild to severe. The most common symptoms include: a high fever, runny nose, sore throat, muscle pains, headache, coughing, and feeling tired. These symptoms typically begin two days after exposure to the virus and most last less than a week. Three types of influenza viruses affect people, called Type A, Type B, and Type C.

Avian influenza (AIV), for most purposes, refers to the influenza A virus. AIV strains are divided into two types based on their pathogenicity: high pathogenicity (HP) or low pathogenicity (LP). H9N* strains belong to HPAIV.

2. Description of Test

The current AIV-H9-Ab ELISA kit is designed to detect AIV H9 subtype specific antibody induced by AIV infection in avian species based on double antigen captured sandwich ELISA. The 96well microtiter plate was precoated with recombinant hemagglutinin (h7) protein. During testing, samples are added into the microplate wells, in which the precoated antigen will capture the AIV H5 antibody in sample and formed antigen-antibody complex. None specific antibody are discarded by a washing step. Then HRP conjugated secondary antibody is added into each well. After another washing step to remove unreacted conjugate, substrate is added and a blue color will be developed if AIV H9 antibody is present. The enzyme reaction is stopped and the OD450nm value is measured. The measured intensity is positively proportional to the amount of antibody present in the sample.

This ELISA kit can be used to detect AIV H9 subtype specific antibody level in **serum**.

3. Precautions

- Store the kit at 2-8℃, check the lot number and expiration date before use.
- Bring the test kit to room temperature before use. For example, take it out from the cold storage and put at room temperature for at least 30min.
- The stop solution in the kit is acidic, please make sure do not touch it with your hand or skin.
- The component of the kit is noninfectious, but the field sample shall be treated as potentially infectious. Please handle all these materials properly according to your lab regulations.
- After experiment, all lab materials shall be handled properly according to local regulations.

4. Limitations of Test

This ELISA kit is currently designed for veterinary use. We recommend validating in your own lab with different methodologies to confirm the performance. If it is not used for the mentioned purpose, please contact us for help.

5. Reagent Provided

The kit contains the following items.

Item No.	Description	Quantity
1	Microplate pre-coated with recombinant H9 protein	1 X 96 wells
2	Positive Control	1 X 1 ml
3	Negative Control	1 X 1 ml
4	Enzyme Conjugate	1 X 25 ml
5	TMB Substrate	1 X 25 ml
6	Stop Solution	1 X 20 ml
7	25X Wash Buffer	1 X 30 ml
8	Sample Buffer	1 X 30 ml
9	Kit Instruction	1set

Note: stop solution (0.5M / 2M sulfuric acid) is not included for safety purpose, which shall be prepared by the users.

6. Instrument Required

- ELISA reader with 450nm/630nm(optional)
- Micropipette 20-200ul, 100-1000ul
- Micropipette Multi-Channel 50-300ul

7. Reagent Preparation

- **Wash buffer**: dilute the 25xWash buffer provided in the kit with deionized water in the volume ratio of 1:24. For example, 1ml 25xWash buffer + 24ml deionized water
  The diluted wash buffer can be stored at 2~8°C for 3 days.
  No other reagent is required. Please remember to return all kit component to room temperature before use.

8. Sample Preparation

- **Serum:** take the blood to be tested and centrifuge it at 4000 r/min for 10 minutes to collect the supernatant.Dilute serum with sample buffer in the volume ratio of 1:100, for example, 2ul serum + 198ul sample buffer, mix thoroughly.

9. Assay Procedure

1) Make sure the kit and all test samples are returned to room temperature before use. Shake each reagent gently before adding into the well.
2) Open the kit, read the kit instruction carefully to make sure all technical points are understood clearly.
3) Take the microplate from the zip-bag, and take needed microwells, store the rest into the zip-bag. Make marks of the plate layout. Running the test in **duplicated** wells is recommended to minimize operational error.
4) Add Positive control: add 100ul positive control into the wells.
5) Add Negative control: add 100ul negative control into the wells.
6) Add Sample: add 100ul diluted serum sample into the wells.
7) Incubation: cover the plate with plate cover and incubate at 37 °C for **25min**.
8) Washing: pour the liquid out from the wells and wash with wash buffer (300ul per well) for 4~5 times. Tap the residue liquid against absorbent paper to make sure the plate is dry after washing.
9) Add enzyme conjugate: add 100ul of HRP enzyme conjugate into each well. Cover the plate

again and then incubate at 37°C again for **25min**.

10) Washing: repeat the washing step again.

11) Add substrate: add the TMB substrate into each well, 100ul per well. Cover the plate again and then incubate at room temperature again for 10min. Color reaction will occur in the plate.

12) Stop the reaction: add 50ul stop solution into each well, the color will turn yellow from blue.

13) Read the plate: using ELISA reader to read the plate at 450nm. If possible, 630nm can be used as reference wavelength.

10. Result Determination

1) Calculation of Cut-off value:

Mean OD of Negative Control X 2 = Cut-off value

2) Criteria of Positive and Negative results.

Positive: **Mean OD of Sample ≥ Cut-off value**

Negative: **Mean OD of Sample < Cut-off value**

11. Performance of Test

According to field test with over 204 samples, the sensitivity of the kit is 95%, and the specificity of the kit is 96.2%.

12. Storage and expiration

The kit shall be store at 2-8°C, avoid direct sunlight.

The valid period is 12 months.

Genprice Inc.

6017 Snell Ave, Suite 357, San Jose, CA. 96123

Phone/Fax: (408) 780-0908 | E: sales@genprice.com / info@gentaur.com