

## Chicken Avian Influenza H5 Virus Antibody (AIV-H5-Ab) ELISA Kit

**Catalog No:** abx364811

**Size:** 96T

**Range:** Qualitative

**Sensitivity:** Qualitative

**Storage:** Store at 2-8°C for 6 months.

**Application:** The qualitative detection of AIV-H5-Ab in Chicken serum, plasma and yolk.

**Principle of the Assay:** This kit is based on competitive enzyme-linked immuno-sorbent assay technology. An antigen is pre-coated onto a 96-well plate. Controls, test samples, and antibody detection reagent are added to the wells and incubated. A competitive inhibition reaction takes place between the AIV-H5-Ab present in the sample and the antibody detection reagent for the pre-coated antigen. The HRP-conjugated reagent is then added, and the whole plate is incubated. Unbound conjugates are removed using wash buffer at each stage. TMB substrate is used to quantify the HRP enzymatic reaction. After TMB substrate is added, only wells that contain sufficient AIV-H5-Ab will produce a blue coloured product, which then changes to yellow after adding the acidic stop solution. The intensity of the yellow colour is inversely proportional to the AIV-H5-Ab amount present in the sample. The Optical Density (OD) is measured spectrophotometrically at 450 nm in a microplate reader, from which the presence of AIV-H5-Ab can be determined.

### Kit Components

- Pre-coated 96-Well Microplate: 12 x 8
- Wash Buffer: (20X) 40 ml
- Positive Control: 1 ml
- Negative Control: 1 ml
- Detection Reagent A: 6 ml
- Detection Reagent B: 11 ml
- TMB Substrate A: 6 ml
- TMB Substrate B: 6 ml
- Stop Solution: 6 ml
- Plate Sealer: 4
- Hermetic Bag: 1

### Materials Required But Not Provided

- 37°C incubator
- Multi and single channel pipettes and sterile pipette tips
- Squirt bottle or automated microplate washer
- 1.5 ml tubes
- Distilled water
- Absorbent filter papers
- 100 ml and 1 liter graduated cylinders
- Microplate reader (wavelength: 450 nm, or 450/630 nm)
- ELISA Shaker

## Protocol

### A. Sample Preparation

Analyse immediately or store samples at 2-8°C (within 24 hrs). For long term storage, aliquot and store at -20°C or -80°C. Avoid multiple freeze-thaw cycles.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 1 hr. Centrifuge at approximately 1000 × g for 20 mins. If precipitate appears, centrifuge again. Assay immediately or aliquot and store at -20°C or -80°C.
- **Plasma:** Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 mins at 1000 x g, within 30 mins of collection. If precipitate appears, centrifuge again. Avoid hemolytic samples.
- **Yolk:** Add 2 ml of physiological saline solution to 2 ml of fresh yolk. Mix thoroughly, then centrifuge at 3000 RPM for 15 minutes. Take the supernatant and analyze immediately.

### Notes:

- **Analyse samples at a 5-fold (1/10) dilution using 1X Wash Buffer (i.e. add 10 µl of sample to 40 µl of 1X Wash Buffer).**
- Store frozen samples undiluted. Once ready to analyse, thaw samples and dilute.
- Fresh samples, or recently obtained samples, are recommended to prevent protein degradation and denaturation that may lead to erroneous results.
- NaN<sub>3</sub> cannot be used as a test sample preservative, since it inhibits HRP.
- If possible, prepare solid samples using sonication and/or homogenization, as lysis buffers may (on occasion) interfere with the kit's performance.
- If a sample is not indicated in the manual's applications, a preliminary experiment to determine the suitability of the kit will be required.

### B. Reagent Preparation

**Wash Buffer:** Dilute the concentrated Wash buffer 20-fold (1/20) with distilled water (i.e. add 20 ml of concentrated wash buffer into 380 ml of distilled water). If crystals have formed in the concentrated Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

### C. Assay Protocol

Equilibrate the kit components and samples to room temperature prior to use. It is recommended to measure in duplicate.

1. Set 2 positive and 2 negative control, test sample and blank (zero) wells on the pre-coated plate respectively, and record their positions. *Add solutions to the bottom of each well without touching the side walls. Pipette the controls and samples up and down to mix before adding to the wells. Avoid foaming or bubbles.*
2. Aliquot 50 µl of negative control and 50 µl of positive control into the respective wells. Leave one well as the blank (zero) well.
3. Aliquot 50 µl of appropriately diluted sample into the test sample wells. Gently tap the plate to mix, or use a microplate shaker.
4. Aliquot 50 µl of Detection Reagent A working solution into each well. Cover the plate with a plate sealer and incubate in the dark for 30 minutes at 37°C.
5. Remove the cover and discard the solution. Wash the plate 3 times with 1X Wash Buffer. *Fill each well completely with Wash buffer (300 µl) using a multi-channel Pipette or autowasher (1-2 mins soaking period is recommended). Complete removal of liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean absorbent paper towels.*
6. Aliquot 100 µl of Detection Reagent B working solution into each well. Cover the plate with a plate sealer and incubate in the dark for 30 mins at 37°C.
7. Remove the cover, discard the solution, and repeat the wash process as described above, 5 times.
8. Aliquot 50 µl of TMB Substrate A and 50 µl of TMB Substrate B into each well. Cover the plate with the plate sealer. Gently tap the plate to mix thoroughly. Incubate at 37°C for 10-20 minutes. The incubation time is for reference only, the optimal time should be determined by end user. Avoid exposure to light.
9. Aliquot 50 µl of Stop Solution into each well. It is important that the Stop Solution is mixed quickly and uniformly throughout the microplate to inactivate the enzyme completely.

# Instructions for Use

Version: 1.0.1

Revision date: 28 Feb 2022



10. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the OD at 450 nm immediately.
11. OPTIONAL: measure the absorbance at 630 nm and subtract these values from the 450 nm values for analysis. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles.

## Data Analysis:

- Mean OD of the Negative Control should be  $\geq 1.0$ .
- Mean OD of the Positive Control should be  $\leq (50\% \times \text{Mean OD of the Negative Control})$ .
- Percentage Inhibition (PI) =  $[ 1 - (\text{Mean OD of Sample} / \text{Mean OD of Negative Control}) ] \times 100\%$

If PI of Samples  $< 50\%$ , the test samples are considered negative.

If PI of Samples  $\geq 50\%$ , the test samples are considered positive.

## Precautions:

- Before using the kit, centrifuge the tubes to bring down the contents trapped in the lid.
- Do not leave the wells uncovered for extended periods between incubations. The addition of reagents for each step should not exceed 10 mins.
- Ensure that the plate is properly sealed or covered during the incubation steps, and that the time and temperature are controlled.
- Do not reuse pipette tips and tubes.
- Do not use expired components, or components from a different kit.
- The TMB substrate should be used under sterile conditions, and light exposure should be minimised. Unused substrate should be colorless, or a very light yellow in appearance. Do not discard any residual solution back into the vial.
- Please note that this kit is optimised for detection of native samples, rather than recombinant proteins or synthetic chemicals. We are unable to guarantee detection of recombinant proteins, as they may have different sequences or tertiary structures to the native protein.
- This kit is for research use only and the results are for reference only. It is recommended to use this kit in conjunction with another detection method

For Reference Only