Signosis
Innovative Plate Assay Solutions

Mouse anti-Influenza HA ELISA Kit

Catalog Number EA-7050 **(For Research Use Only)**

Introduction

Hemagglutinin (HA) is a surface glycoprotein on the influenza virus and plays a central role in viral infection and immunity. HA is significant because seasonal influenza vaccines are made by using HA proteins from predicted circulating strains.

Principle of the assay

Each well of the plate is coated with OVA, which detects anti-OVA antibodies in serum or plasma samples. After the anti-TNP antibodies binds to the plate and the samples are washed away, an anti-mouse IgE-HRP conjugate is added to the wells to form a complex with the antibodies bound to the plate. After incubation, the wells are washed to remove unbound HRP-labeled antibodies. The HRP substrate, TMB, is then added which results in the formation of a blue color in the presence of HRP. The reaction is then terminated with the addition of Stop Solution, resulting in a yellow color, which is measured spectrophotometrically at an absorbance of 450 nm. The concentrations of anti-OVA antibodies in the sample are directly proportional to the color intensity of the well.

Materials provided with the kit

Component	Qty	Store at
96-Well Plate coated with OVA	1	4°C
HRP-labeled antibody against mouse IgE antibodies	200 µL	-20°C
1X Diluent Buffer	50 µL	4°C
5X Assay Wash Buffer	40 mL	4°C
Substrate	40 mL	4°C
Stop Solution	10 mL	4°C

Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer
  - 40ml 5x Assay wash buffer
  - 160ml ddH_2O.
- Dilute 100 times of HRP-labeled antibody with 1X Diluent buffer.

Sample preparation before starting experiment

- For **cell culture medium samples**, add 100µl directly to the well.
- For **cell lysate samples**, use cell lysis buffer (Catalog# EA-0001). Follow protocol in Cell Lysate Buffer User Manual.
- For **serum or plasma samples**, we recommend a 1:10 dilution with 1X diluent buffer, for example, add 80ul sample in 720ul 1X diluent buffer. When serum-containing conditional media is required, be sure to use serum as control.

Assay procedure

1. Use the desired number of wells from the plate. Make sure the rest of wells are well sealed.
2. Sample assay:
Apply each sample in the plate, 100ul per well and incubate for 1-2 hour at room temperature with gentle shaking.
3. Aspirate each well and wash by adding 200µl of 1X Assay wash buffer. Repeat the process three times for a total of three washes. Completely remove liquid at each wash. After the last wash, remove any remaining liquid by inverting the plate against clean paper towels.
4. Add 100µl of diluted HRP-labeled antibody to each well and incubate for 1 hour at room temperature with gentle shaking.
5. Repeat the aspiration/wash as in step 4.
6. Add 100µl substrate to each well and incubate for 10-30 minutes.

Note: Substrate incubation time may vary due to different antibodies reactivity. Stronger signals (Strong blue color) could be stopped earlier. Weaker signals can be incubated for longer than 30 minutes. **Always stop all the sample reactions in the plate at the same time.**

7. Add 50µl of Stop solution to each well. The color in the wells should change from blue to yellow.
8. Determine the optical density of each well with a microplate reader at 450 nm.