Influenza B (Pan) Hemagglutinin ELISA Development Kit

Catalog Number: IT-E3Ag-Influenza (B/HA)-Pan

BACKGROUND:

Influenza B virus, member of the Orthomyxoviridae family, causes human influenza, or commonly known as "flu". Although it has limited host range and lower mutation rate compared to influenza A virus, antigenic drifting in Hemagglutinin of influenza B virus often occurs. However, conserved antigenic sites in Hemagglutinin of influenza B virus have been identified, including sites targeted by potent and broad neutralizing anti- influenza B virus antibodies discovered to date. "Influenza B Hemagglutinin ELISA Development Kit" utilizes antibodies recognizing conserved epitopes in Hemagglutinin with high affinity, thus is suitable for detecting sub-nanogram level of influenza B Hemagglutinin in biological samples, including quantitating Hemagglutinin protein in seasonal flu vaccine preparation.

PRODUCT DESCRIPTION:

"Influenza B Hemagglutinin ELISA Development Kit" contains the key components required for the quantitative analysis of Influenza B Hemagglutinin in cell culture supernatants, serum, and other biological samples within the range of 0.01 - 10ng/ml in a sandwich ELISA format. The components supplied in this kit are sufficient to perform the assay in approximately 500 ELISA plate wells.

REAGENTS PROVIDED

Capture Antibody: 100µl of 1mg/ml anti-Influenza B Hemagglutinin monoclonal antibody.

Protein Standard: 50µl of 50µg/ml recombinant Hemagglutinin (ΔTM)(B/Wisconsin/01/2010).

Detection Antibody: 50µl of biotinylated monoclonal antibody against Influenza B Hemagglutinin.

Streptavidin-HRP Conjugate: 50µl of HRP- conjugated streptavidin.

RECOMMENDED MATERIALS (not included in the kit)*

ELISA 96-well plates: Corning Prod # 3590 or equivalent plate

Block Buffer: 5% skim milk in PBS

Wash Buffer: 0.05% Tween-20 in PBS

Diluent: 0.05% Tween-20, 0.1% skim milk in PBS

Substrate: TMB Peroxidase Substrate

Stop Solution: 2N Sulfuric Acid

***Alternatively, purchase Immune Tech ELISA Plate/Buffer/Substrate Kit: Cat* #IT-200-002.**

Influenza B (Pan) Hemagglutinin ELISA Development Kit

Catalog Number: IT-E3Ag-Influenza (B/HA)-Pan

PLATE PREPARATION

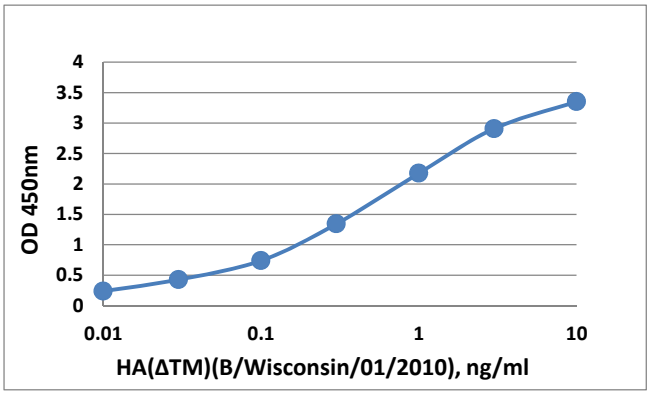
1. **Plate coating:**
   a. For each 96-well plate, dilute 20µl of capture antibody with 10.5ml of PBS.
   b. Add 100µl to each well immediately.
   c. Seal the plate and incubate overnight at 4°C.
2. **Washing**
   a. Remove the coating reagent by aspirating or decanting.
   b. Invert the plate and blot on the paper towel.
3. **Blocking and Washing**
   a. Add 300µl of block buffer to each well.
   b. Incubate for at least 1 hour at room temperature.
   c. Aspirate the wells to remove liquid and wash the plate 4 times with 300µl of wash buffer per well.

ASSAY PROCEDURE

1. **Standard/Sample preparation:**
   a. Dilute standard with Diluent to the following eight concentrations
   --31.6ng/ml,
   --10ng/ml
   --3.16ng/ml
   --1ng/ml
   --0.316ng/ml
   --0.1ng/ml
   --0.0316ng/ml
   --0ng/ml
   b. Make a serial dilution of testing samples with Diluent
   c. Immediately, add 100µl of standard and sample to each well in triplicate.
   d. Incubate at 37°C for 1 hour.
2. **Detection:**
   a. Aspirate and wash plate 4 times.
   b. Dilute 10µl of detection antibody with 10.5ml of PBS.
   c. Add 100µl into each well.
   d. Incubate at 37°C for 1 hour.
3. **Streptavidin Peroxidase:**
   a. Aspirate and wash plate 4 times.
   b. Dilute 5µl of Streptavidin-HRP conjugate with 10.5ml of PBS.
   c. Add 100µl into each well.
   d. Incubate at 37°C for 45 minutes.
4. **Substrate/Stop:**
   a. Aspirate and wash plate 4 times.
   b. Add 100µl of TMB Peroxidase Substrate into each well.
   c. Incubate at 37°C for 25 minutes.
   d. Add 100µl of stop solution to each well.
5. **Read:** Determine the optical density of each well within 30 minutes, using a microplate reader set to 450nm.

6. **Analysis:**
   a. Average the triplicate readings from the standard, control, and sample
   b. Subtract the average zero standard optical density
   c. Generate a standard curve by applying the data using computer software capable of generating a four parameter logistic (4-PL) or other curve-fit.
   d. The Influenza B Hemagglutinin concentration in sample can be determined by regression analysis.
   e. If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor.

Figure 1. Standard curve with serial dilution of protein standard.



Reference
John R. Crowther. The ELISA Guidebook (Methods in Molecular Biology), Humana Press, 2000.