Elite™ Hemagglutinin (H3N2)(A/Brisbane/10/2007) ELISA Assay Kit

CATALOG NUMBER: CA-I307, 200 assays (2x 96-well plates)

Description

Hemagglutinin (HA)(H3N2)(A/Brisbane/10/2007) ELISA Assay Kit contains the key components required for the quantitative analysis of Hemagglutinin (HA(H3N2)(A/Brisbane/10/2007) concentrations in cell culture supernatants and serum within the range of 0.125-8 ng/ml in a sandwich ELISA format. A pair of matched monoclonal antibodies has been selected as capture antibody and detection antibody. The components supplied in this kit are sufficient to assay HA(H3N2)(A/Brisbane/10/2007) in two 96-well ELISA plates.

Kit Components

- **Component A:** Capture Antibody (anti-HA(H3N2)(A/Brisbane/10/2007) monoclonal antibody)
  50 µl (1 mg/ml)
- **Component B:** HA Standard (recombinant HA (H3N2)(A/Brisbane/10/2007) protein)
  25 µl (50 µg/ml)
- **Component C:** Detection Antibody (biotinylated anti-HA(H3N2)(A/Brisbane/10/2007) monoclonal antibody)
  25 µl
- **Component D:** HRP-Conjugated Streptavidin
  25 µl
- ***Component E:** TMB Solution A (3,3',5,5'- tetramethylbenzidine) (light sensitive!)
  15 ml
- ***Component F:** TMB Solution B (H_2O_2) (light sensitive!)
  15 ml
- ***Component G:** TMB Stop Solution
  30 ml

***Note: Components EFG** are complimentary to customers within the USA. These 3 components are not included in the kit for customers outside the USA as they are considered “hazardous” and restricted for import in many countries. Please see the **Appendix** for solutions. The TMB reagents are commercially available from local life science reagent sellers.

Materials Required but not Provided

- 96-well microtiter plates designed for ELISA assay
- PBS
- Coating Buffer: 0.05 M Carbonate-Biocarbonate, pH 9.6
- Blocking Buffer: 5% milk in PBS
- Washing Buffer: 0.05% Tween-20 in PBS
- Dilution Solution: 0.05% Tween-20 and 0.5% milk in PBS

Storage

Keep in 4 °C and avoid exposure to light; do not freeze!

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Assay Protocol

1. **Plate Preparation:**

1.1 For each 96-well microtiter plate, dilute 20 µl of Capture Antibody (**Component A**) with 10.5 ml of Coating Buffer to prepare a coating solution. Immediately add 100 µl of the coating solution to each well. Seal the plate and incubate overnight at 4 °C.

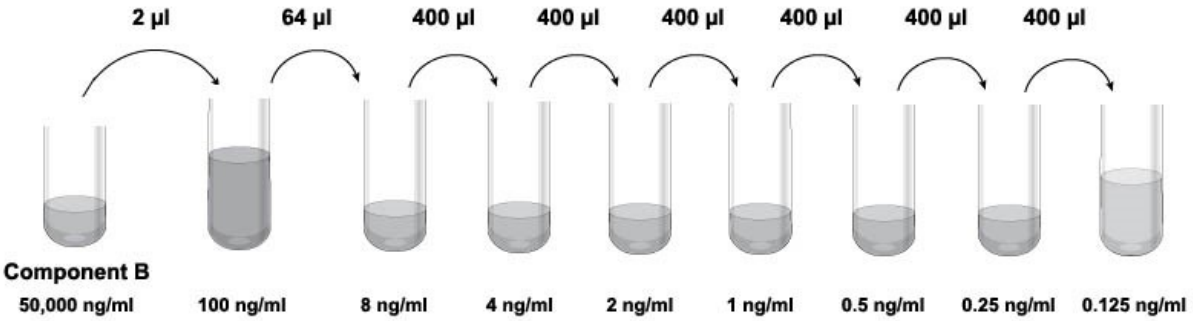
1.2 Remove the coating solution and wash the plate twice with 200 µl PBS. Invert the plate and blot it briefly with clean paper towel.

1.3 Add 300 µl of Blocking Buffer to each well. Incubate for at least 1 hour at room temperature.

1.4 Aspirate to remove Blocking Buffer and wash the plate 4 times with 300 µl of Washing Buffer per well.

2. **Run ELISA Assay:**

2.1 Standard/Sample: Dilute the HA Standard (**Component B**) with PBS Solution to eight concentrations (8 ng/ml, 4 ng/ml, 2 ng/ml, 1 ng/ml, 0.5 ng/ml, 0.25 ng/ml, 0.125 ng/ml, and 0 ng/ml). Immediately add 100 µl of Standard and sample to each well in triplicate. Incubate at room temperature for at least 1 hour.



2.2 Detection: Aspirate and wash plate 4 times. Dilute 10 µl of Detection Antibody (**Component C**) with 10.5 ml of Dilution Solution to prepare a detection solution. Add 100 µl of the detection solution into each well. Incubate at room temperature for at least 1 hour.

2.3 Streptavidin Peroxidase: Aspirate and wash plate 4 times. Dilute 10 µl of HRP-Conjugate Streptavidin (**Component D**) with 10.5 ml of Dilution Solution. Add 100 µl into each well. Incubate at room temperature for 30 minutes.

2.4 TMB Peroxidase Substrate Solution Preparation: Mix equal volumes of TMB Solution A (**Component E**) and TMB Solution B (**Component F**) in a clean, preferably HDPE, polypropylene or glass container immediately prior to use at room temperature.

Note: *For one 96-well plate, prepare 12 ml TMB Peroxidase Substrate Solution by mixing 6 ml* ***Component E*** *and* ***Component F***

2.5 TMB Reaction: Aspirate and wash plate 4 times with Washing Buffer. Add 100 µl of TMB Peroxidase Substrate Solution (from Step 2.4) into each well. Incubate at room temperature for 20 minutes.

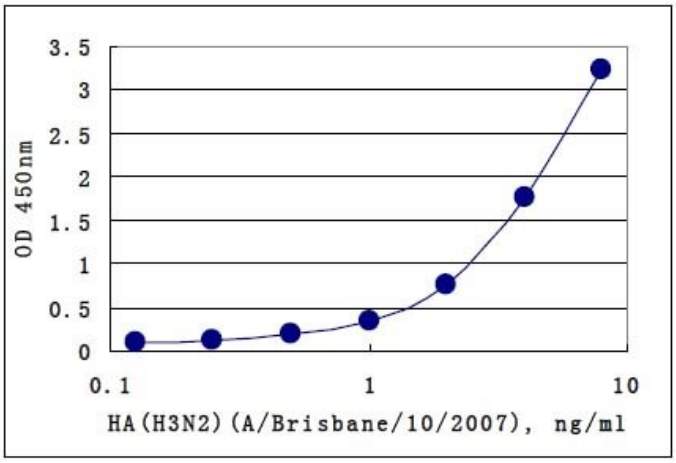
2.6 TMB Reaction Termination: Add 100 µl of TMB Stop Solution (**Component G**) to each well. This stop solution will halt color development and will turn the TMB Substrate from blue into yellow.

2.7 Read: Determine the optical density of each well within 30 minutes using a microplate reader set to 450nm.

2.8 Analysis: Average the triplicate reading for each standard, control, and sample, then subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four-parameter logistic (4-PL) or other curve-fit.

The HA (H3N2)(A/Brisbane/10/2007) concentration in sample can be determined by regression analysis. If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor.

(See the plot on the next page)



Appendix: TMB Solutions for ELISA Assay

1. **TMB stock solution**: weigh 10 mg of TMB (3,3',5,5'- tetramethylbenzidine, light sensitive!) and dissolve it in 10 mL of DMSO, stable for one year at 4 °C.

2. **Working substrate-chromogen solution for ELISA**: dilute 1 mL of TMB-DMSO stock solution (from Step 1) with 9 mL of phosphate-citrate buffer pH 5.2, and add 2 microlitre of hydrogen peroxide (30%).

3. Add 100 microlitre of Working Substrate-Chromogen Solution to each well of 96-well ELISA assay plate. Incubate at room temperature for 10-30 minutes

4. Add 100 microlitre of **TMB Stop Solution (1M H_3PO_4)** to stop color reaction.