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- Trockeneiszuschlag
- Gefahrgutzuschlag
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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)
  15 ml
- **Component F:** TMB Solution B (H_2O_2)
  15 ml
- **Component G:** TMB Stop Solution
  30 ml

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 ^{o}C and avoid exposure to light; do not freeze!

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 ^{o}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:**

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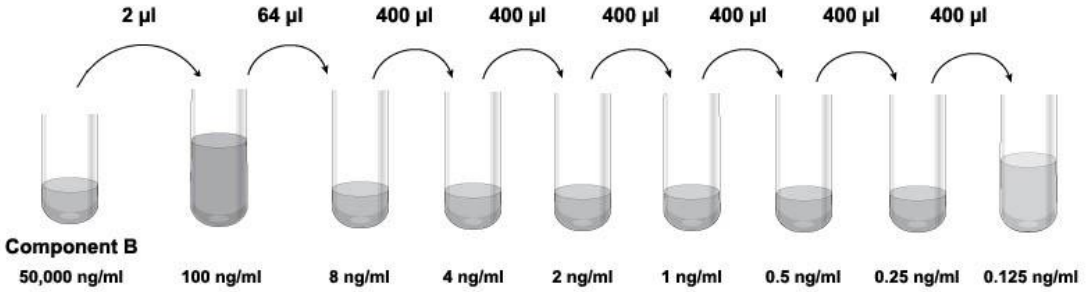
2.1 Standard/Sample: Dilute the HA Standard (**Component B**) with PBS Solution to eight concentrations (8 ng/ml, 4 ng/ml, 2 ng/ml, 1 ng/ml, 0.5 ng/ml, 0.25 ng/ml, 0.125 ng/ml, and 0 ng/ml). Immediately add 100 µl of Standard and sample to each well in triplicate. Incubate at room temperature for at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

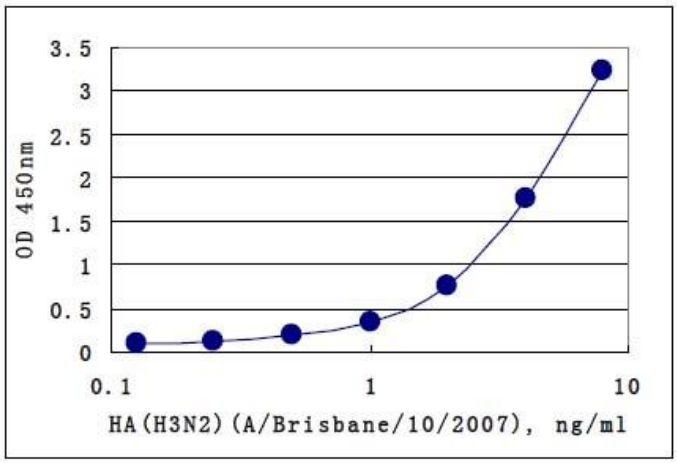
*Note: For one 96-well plate, prepare 12 ml TMB Peroxidase Substrate Solution by mixing 6 ml **Component E** and **Component F**.*

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.



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