Immune Technology Corp.
The Resource for Virology Research

HA(H1N1)(A/California/07/2009)
Hemagglutinin ELISA Development Kit
Catalog Number: IT-E3Ag-2009H1N1-California

Description: HA(H1N1)(A/California/07/2009) Hemagglutinin ELISA Development Kit contains the key components required for the quantitative analysis of HA(H1N1)(A/California/07/2009) Hemagglutinin concentrations in cell culture supernatants and serum within the range of 0.3-300ng/ml in a sandwich ELISA format. The components supplied in this kit are sufficient to assay HA(H1N1)(A/California/07/2009) Hemagglutinin in approximately 500 ELISA plate wells.

REAGENTS PROVIDED

Capture Antibody: 100µl of 1mg/ml anti-HA(H1N1)(A/California/07/2009) Hemagglutinin monoclonal antibody.
HA(H1N1)(A/California/07/2009) Standard: 50µl of 50µg/ml recombinant HA(H1N1)(A/California/07/2009).
Detection Antibody: 50µl of polyclonal antibody against to HA((H1N1)(A/California/07/ 2009).
Anti-Rabbit-IgG-HRP Conjugate: 50µl of HRP-conjugated anti-rabbit-IgG.

RECOMMENDED MATERIALS & SOLUTIONS*

ELISA 96-well plates (Corning Prod # 3590 or equivalent plate)
Block Buffer: 5% milk in PBS
Wash Buffer: 0.05% Tween-20 in PBS
Diluent: 0.05% Tween-20, 0.5% milk in PBS
Substrate: TMB Peroxidase Substrate
Stop Solution: 2N Sulfuric Acid
****or purchase ImmuneTech ELISA Plate/Buffer/Substrate Kit: Cat #IT-200-002.***

PLATE PREPARATION

1. For each 96-well plate, dilute 20µl of capture antibody with 10.5ml of PBS. Immediately, add 100µl to each well. Seal the plate and incubate overnight at 4°C.
2. Remove the coating reagent by aspirating or decanting. Invert the plate and blot on the paper towel.
3. Add 300µl of block buffer to each well. Incubate for at least 1 hour at 37°C.
4. Aspirate the wells to remove liquid and wash the plate 4 times with 300µl of wash buffer per well.

ASSAY PROCEDURE

1. **Standard/Sample:** Dilute standard with Diluent to eight concentrations (300ng/ml, 100ng/ml, 30ng/ml, 10ng/ml, 3ng/ml, 1ng/ml, 0.3ng/ml, 0ng/ml). Immediately, add 100µl of standard and sample to each well in triplicate. Incubate at 37°C for 1 hour.
2. **Detection:** Aspirate and wash plate 4 times. Dilute 10µl of detection antibody with 10.5ml of Diluent. Add 100µl into each well. Incubate at 37°C for 1 hour.
3. **Anti-Rabbit-IgG Peroxidase:** Aspirate and wash plate 4 times. Dilute 10µl of anti-rabbit-IgG-HRP conjugate with 10.5ml of Diluent. Add 100µl into each well. Incubate at 37°C for 45 minutes.
4. **Substrate/Stop:** Aspirate and wash plate 4 times. Add 100µl of TMB Peroxidase Substrate into each well. Incubate at 37°C for 20 minutes. Then 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:** Average the triplicate reading for each standard, control, and sample and 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(H1N1)(A/California/07/2009) 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.