Immune Technology Corp.

The Resource for Virology Research

HA(H14N5)(A/Mallard/Astrakhan/263/1982)
Antigen ELISA Development Kit
Catalog Number: IT-E3Ag-H14N5-Mallard/Astrakhan/263/1982

Description: HA(H14N5)(A/Mallard/Astrakhan/263/1982) antigen ELISA Development Kit contains the key components required for the quantitative analysis of HA(H14N5)(A/Mallard/Astrakhan/263/1982) antigen concentrations in cell culture supernatants and serum within the range of 1-1000ng/ml in a sandwich ELISA format. The components supplied in this kit are sufficient to perform the assay in five 96-well ELISA plates.

REAGENTS PROVIDED

Capture Antibody: 100µl of 1mg/ml anti-HA(H14N5)(A/Mallard/Astrakhan/263/1982) monoclonal antibody.
HA(H14N5)(A/Mallard/Astrakhan/263/1982)
Standard: 50µl of 50µg/ml recombinant HA(H14N5)(A/Mallard/Astrakhan/263/1982).
Detection Antibody: 50µl of biotinylated monoclonal antibody against HA(H14N5)(A/Mallard/Astrakhan/263/1982).
Streptavidin-HRP Conjugate: 50µl of HRP-conjugated streptavidin.

RECOMMENDED MATERIALS & SOLUTIONS*

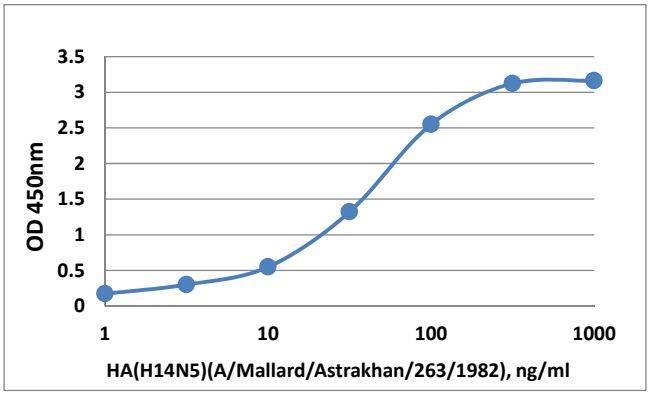
ELISA 96-well plates (Corning Prod # 3590 or equivalent plate)
Block Buffer: 5% milk in PBS
Wash Buffer: 0.05% Tween-20 in PBS
Diluent: 0.05% Tween-20, 0.5% milk in PBS
Substrate: TMB Peroxidase Substrate
Stop Solution: 2N Sulfuric Acid
* ***Alternatively, these could be purchased under Cat.# IT-200-002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

PLATE PREPARATION

1. For each 96-well plate, dilute 20µl of Capture Antibody with 10.5ml of 1xPBS to prepare a coating solution. Immediately add 100µl of the coating solution to each well. Seal the plate and incubate overnight at 4°C.
2. Remove the coating solution by aspirating or decanting. Invert the plate and blot it briefly against clean paper towels.
3. Add 300µl of Block Buffer to each well. Incubate at 37°C for 2 hours.
4. Aspirate to remove Block Buffer 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 (1000ng/ml, 316ng/ml, 100ng/ml, 31.6ng/ml, 10ng/ml, 3.16ng/ml, 1ng/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 to prepare a detection solution. Add 100µl of the detection solution into each well. Incubate at 37°C for 1 hour.
3. **Streptavidin Peroxidase:** Aspirate and wash plate 4 times. Dilute 10µl of Streptavidin-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 30 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(H14N5)(A/Mallard/Astrakhan/263/1982) 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.



Reference

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