



**HA(H1N1)(A/South Carolina/1/18)  
Hemagglutinin ELISA Development Kit**  
Catalog Number: IT-E3Ag-H1N1-South Carolina/1/18

**Description:** HA(H1N1)(A/South Carolina/1/18) Hemagglutinin ELISA Development Kit contains the key components required for the quantitative analysis of HA(H1N1)(A/South Carolina/1/18) Hemagglutinin (HA) concentrations in cell culture supernatants and serum within the range of 4-256 ng/ml in a sandwich ELISA format. The components supplied in this kit are sufficient to assay HA(H1N1)(A/South Carolina/1/18) in five 96-well ELISA plates.

**REAGENTS PROVIDED**

**Capture Antibody:** 100 $\mu$ l of 1mg/ml anti-HA(H1N1)(A/South Carolina/1/18) monoclonal antibody.

**HA1(H1N1)(A/South Carolina/1/18) Standard:** 50 $\mu$ l of 50 $\mu$ g/ml recombinant HA(H1N1)(A/South Carolina/1/18).

**Detection Antibody:** 50 $\mu$ l of biotinylated monoclonal antibody against HA1(H1N1)(A/South Carolina/1/18).

**Streptavidin-HRP Conjugate:** 50 $\mu$ l of HRP-conjugated streptavidin.

**RECOMMENDED MATERIALS & SOLUTIONS\***

ELISA 96-well plates (Corning Prod # 3590 or equivalents)

**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.*

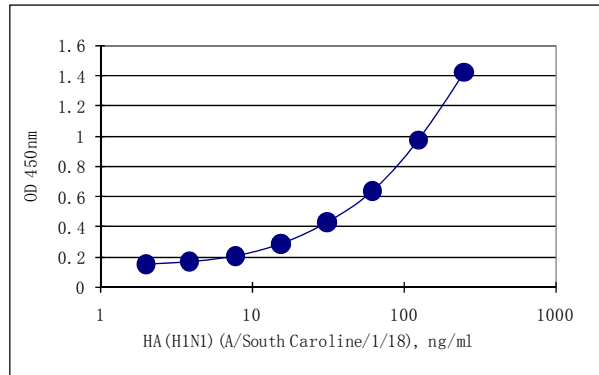
**PLATE PREPARATION**

1. For each 96-well plate, dilute 20 $\mu$ l of Capture Antibody with 10.5ml of 1xPBS to prepare a coating solution. Immediately add 100 $\mu$ l 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 300 $\mu$ l of Block Buffer to each well. Incubate for at least 1 hour at room temperature.

4. Aspirate to remove Block Buffer and wash the plate 4 times with 300 $\mu$ l of Wash Buffer per well.

**ASSAY PROCEDURE**

1. **Standard/Sample:** Dilute the standard with Diluent to eight concentrations (256ng/ml, 128ng/ml, 64ng/ml, 32ng/ml, 16ng/ml, 8ng/ml, 4ng/ml, and 0ng/ml). Immediately add 100 $\mu$ l of Standard and sample to each well in triplicate. Incubate at room temperature for at least 1 hour.
2. **Detection:** Aspirate and wash plate 4 times. Dilute 10 $\mu$ l of Detection Antibody with 10.5ml of Diluent to prepare a detection solution. Add 100 $\mu$ l of the detection solution into each well. Incubate at room temperature for at least 1 hour.
3. **Streptavidin Peroxidase:** Aspirate and wash plate 4 times. Dilute 10 $\mu$ l of Streptavidin-HRP Conjugate with 10.5ml of Diluent. Add 100 $\mu$ l into each well. Incubate at room temperature for 30 minutes.
4. **Substrate/Stop:** Aspirate and wash plate 4 times. Add 100 $\mu$ l of TMB Peroxidase Substrate into each well. Incubate at room temperature for 20 minutes. Then add 100 $\mu$ l 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, 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(H1N1)(A/South Carolina/1/18) 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**

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