**INTENDED USE**

The 2009 H1N1 Influenza (Swine Flu) HA ELISA kit is for the quantitative determination of 2009 H1N1 Influenza (Swine Flu) HA.

This ELISA kit contains the basic components required for the development of sandwich ELISAs. Each kit contains sufficient materials to run ELISAs on five 96-well plates.

**PRINCIPLE OF THE TEST**

The ELISA kit is a solid phase sandwich ELISA (Enzyme-Linked Immunosorbent Assay). It utilizes a monoclonal antibody specific for 2009 H1N1 Influenza (Swine Flu) HA coated on a 96-well plate. Standards and samples are added to the wells, and any 2009 H1N1 Influenza (Swine Flu) HA present binds to the immobilized antibody. The wells are washed and a biotinylated rabbit anti-2009 H1N1 Influenza (Swine Flu) HA monoclonal antibody is then added, producing an antibody-antigen-antibody "sandwich". To produce color in proportion to the amount of 2009 H1N1 Influenza (Swine Flu) HA present in the sample streptavidin-HRP and TMB substrate solution are loaded. The absorbances of the microwell are read at 450nm.

**SPECIFICITY&Sensitivity**

The minimum detectable dose of 2009 H1N1 Influenza (Swine Flu) HA was determined to be approximately 12.2 pg/ml. This is defined as at least three times standard deviations above the mean optical density of 10 replicates of the zero standard. The following hemagglutinin of different influenza virus types and subtypes prepared at 200ng/ml were tested and no cross-reactivity was identified.

**MATERIALS PROVIDED**

Bring all reagents to room temperature before use.

**Capture Antibody**: 0.2 mg/ml of mouse anti-2009 H1N1 Influenza (Swine Flu) HA monoclonal antibody. Dilute to a working concentration of 2 μg/ml in CBS before coating.

**Detection Antibody**: 0.5 mg/ml of rabbit anti-2009 H1N1 Influenza (Swine Flu) HA polyclonal antibody conjugated to horseradish-peroxidase(HRP). Dilute to a working concentration of 2 μg/ml in detection antibody dilution buffer before use.

**Standard**: Each vial contains 45ng of recombinant 2009 H1N1 Influenza (Swine Flu) HA. Reconstitute with 1 ml detection antibody dilution buffer. After reconstitution, store at -20°C to -70°C in a manual defrost freezer. A nine-point standard curve using 2-fold serial dilutions in sample dilution buffer, and a high standard of 780 pg/ml is recommended.

**ANALYTE GENE INFORMATION**

**Gene Name**: HA haemagglutinin [Influenza A virus (A/Puerto Rico/8/1934(H1N1))]

**Official Symbol**: HA

**Synonyms**: HA; hemagglutinin

**GeneID**: 956529

**Protein Refseq**: NP_040980.1

**Chromosome Location**: segment 4

**Pathway**: Assembly of Viral Components at the Budding Site; Budding; Entry of Influenza Virion into Host Cell via Endocytosis; Fusion and Uncoating of the Influenza Virion; Fusion of the Influenza Virion to the Host Cell Endosome; Influenza Infection; Influenza Life Cycle; Influenza Viral RNA Transcription and Replication; Packaging of Eight RNA Segments; Release; Transport of HA trimer; Uncoating of the Influenza Virion; Viral mRNA Translation; Virus Assembly and Release

**SOLUTIONS REQUIRED**

**CBS**: 0.05M Na₂CO₃, 0.05M NaHCO₃, pH9.6, 0.2μm filtered

**TBS**: 25mM Tris, adjust pH to 7.4 by HCl

**Wash Buffer**: 0.05% Tween20 in TBS, pH 7.2-7.4

**Blocking Buffer**: 2% BSA in Wash Buffer

**Sample dilution buffer**: 0.1% BSA in wash buffer, pH7.2-7.4, 0.2μm filtered

**Detection antibody dilution buffer**: 0.5% BSA in wash buffer, pH7.2-7.4, 0.2μm filtered

**Substrate Solution**: To achieve best assay results, fresh substrate solution is recommended

**Substrate stock solution**: 10 mg/ml TMB in DMSO

**Substrate dilution buffer**: 0.05M Na₂HPO₄ and 0.025M citric acid; adjust pH to 5.5

**Substrate working solution**: For each plate dilute 250 μl substrate stock solution in 25ml substrate dilution buffer and then add 80μl 0.75% H₂O₂, mix it well

**Stop Solution**: 2N H₂SO₄
**CALCULATION OF RESULTS**

Calculate the mean absorbance for each set of duplicate standards, controls and samples. Subtract the mean zero standard absorbance from each.

Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.

To determine the concentration of the unknowns, find the unknowns' mean absorbance value on the y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the x-axis and read the concentration. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Alternatively, computer-based curve-fitting statistical software may also be employed to calculate the concentration of the sample.

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**PRECAUTIONS**

The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

**STORAGE**

Detection Antibody should be protected from prolonged exposure to light. Aliquot all other reagents and store at -20°C to -70°C in a manual defrost freezer.

**ELISA PROTOCOL**

**Plate Preparation**

1. Dilute the capture antibody to the working concentration in CBS. Immediately coat a 96-well microplate with 100μL per well of the diluted capture antibody. Seal the plate and incubate overnight at 4°C.
2. Aspirate each well and wash with at least 300μl wash buffer, repeating the process two times for a total of three wash-es. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining wash buffer by inverting the plate and blotting it against clean paper towels.
3. Block plates by adding 300μl of blocking buffer to each well. Incubate at room temperature for a minimum of 1 hour.

**Assay Procedure**

1. Add 100 μl of sample or standards in sample dilution buffer per well. Seal the plate and incubate 2 hours at room temperature.
2. Repeat the aspiration/wash as in step 2 of plate preparation.
3. Add 100 μl of the detection antibody, diluted in antibody dilution buffer, to each well. Seal the plate and incubate 1 hour at room temperature.
4. Repeat the aspiration/wash as in step 2 of plate preparation.
5. Add 200 μl of substrate solution to each well. Incubate for 20 minutes at room temperature (*if substrate solution is not as requested, the incubation time should be optimized*). Avoid placing the plate in direct light.
6. Add 50μl of stop solution to each well. Gently tap the plate to ensure thorough mixing.
7. Determine the optical density of each well immediately, using a microplate reader set to 450nm.

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<th>Concentration (pg/ml)</th>
<th>Zero standard subtracted OD</th>
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<tbody>
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