H1N1 HA ELISA Kit

Prod. No.: DEIA533
Pkg. Size: 5 Plates

INTENDED USE

The H1N1 (A/Puerto Rico/8/1934) HA ELISA kit is for the quantitative determination of H1N1 (A/Puerto Rico/8/1934) 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.

GENERAL DESCRIPTION

Influenza (flu) is a respiratory infection in mammals and birds. This virus is divided into three main types (A, B and C). Influenza A is found in a wide variety of bird and mammal species and is further divided into subtypes based on differences in the membrane proteins hemagglutinin (HA) and neuraminidase (NA). Influenza A H1N1 virus is a subtype of influenza A virus. Some strains of H1N1 are endemic in humans and cause a small fraction of all influenza-like illness and a small fraction of all seasonal influenza. H1N1 strains caused a few percent of all human flu infections in 2004-2005. Other strains of H1N1 are endemic in pigs (swine influenza) and in birds (avian influenza). H1N1 was the most common cause of human influenza (flu) in 2009. In June 2009, the World Health Organization declared the new strain of swine-origin H1N1 as a pandemic. This strain is often called swine flu by the public media. This novel virus spread worldwide and had caused about 17,000 deaths by the start of 2010. Hemagglutinin (HA) is a single-pass type I integral membrane glycoprotein from the influenza virus, and comprises over 80% of the envelope proteins present in the virus particle. In natural infection, inactive HA is matured into HA1 and HA2 outside the cell by one or more trypsin-like, arginine-specific endoprotease secreted by the bronchial epithelial cells. Binding of HA to sialic acid-containing receptors on the surface of its target cell brings about the attachment of the virus particle to the cell and forms a endosome. Low pH in endosomes induce an irreversible conformational change in HA2, releasing the hydrophobic portion “fusion peptide”. After which, virus penetrates the cell and pours its contents including the RNA genome into the cytoplasm mediated by fusion of the endocytosed virus particle’s own membrane and the endosomal membrane. Hemagglutinin plays a major role in the determination of host range restriction and virulenc

PRINCIPLE OF THE TEST

The ELISA kit is a solid phase sandwich ELISA (Enzyme-Linked Immunosorbent Assay). It utilizes a monoclonal antibody specific for H1N1 (A/Puerto Rico/8/1934) HA coated on a 96-well plate. Standards and samples are added to the wells, and any H1N1 (A/Puerto Rico/8/1934) HA present binds to the immobilized antibody. The wells are washed and a horseradish peroxidase conjugated mouse anti- H1N1 (A/Puerto Rico/8/1934) HA monoclonal antibody is then added, producing an antibody-antigen-antibody “sandwich”. The wells are again washed and TMB substrate solution is loaded, which produces color in proportion to the amount of H1N1 (A/Puerto Rico/8/1934) HA present in the sample. To end the enzyme reaction, the stop solution is added and absorbances of the microwell are read at 450 nm.

REAGENTS PROVIDED

Bring all reagents to room temperature before use.
1. Capture Antibody – 0.3 mg/mL of mouse anti-H1N1 (A/Puerto Rico/8/1934) HA monoclonal antibody. Dilute to a working concentration of 1 µg/mL in CBS before coating.
2. Detection Antibody – 0.5 mg/mL mouse anti-H1N1 (A/Puerto Rico/8/1934) HA monoclonal antibody conjugated to horseradish-peroxidase (HRP). Dilute to working concentration of 2 µg/mL in detection antibody diluteion buffer before use.
3. Standard – Each vial contains 50 ng of recombinant anti-H1N1 (A/Puerto Rico/8/1934) HA. Reconstitute with 1 mL detection antibody dilution buffer. After reconstitution, store at -20℃ to -70℃ in a manual defrost freezer . A seven-point standard curve using 2-fold serial dilutions in sample dilution buffer, and a high standard of 4 ng/mL is recommended.
4. CBS - 0.05M Na_2CO_3, 0.05M $NaHCO_3$, pH 9.6, 0.2 µm filtered
5. TBS - 20 mM Tris, 150 mM NaCl, pH 7.4
6. Wash Buffer - 0.05% Tween20 in TBS, pH 7.2 - 7.4
7. Blocking Buffer - 2% BSA in Wash Buffer
8. Sample dilution buffer - 0.1% BSA in wash buffer, pH 7.2 - 7.4, 0.2 µm filtered
9. Detection antibody dilution buffer - 0.5% BSA in wash buffer, pH 7.2 - 7.4, 0.2 µm filtered
10. Substrate Solution: To achieve best assay results, fresh substrate solution is recommended
11. Substrate stock solution - 10 mg/ml TMB (Tetramethylbenzidine) in DMSO
12. Substrate dilution buffer - 0.05M Na_2HPO_4 and 0.025M citric acid ; adjust pH to 5.5
13. Substrate working solution - For each plate dilute 250 µl substrate stock solution in 25ml substrate dilution buffer and then add 80 µl 0.75% H_2O_2, mix it well
14. Stop Solution - 2 N H_2SO_4

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STORAGE

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

ASSAY PROCEDURE

1. 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℃.
2) Aspirate each well and wash with at least 300μl wash buffer, repeating the process two times for a total of three washes. 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 300 μL of blocking buffer to each well. Incubate at room temperature for a minimum of 1 hour.
4) Repeat the aspiration/wash as in step 2. The plates are now ready for sample addition.

2. Assay Steps

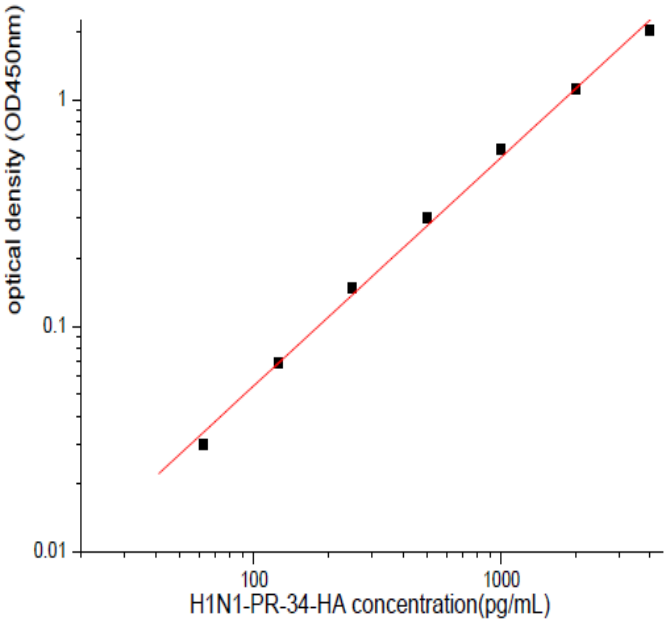
1) Add 100 μL of sample or standards in sample dilution buffer per well. Seal the plate and incubate 2 hours at room temperature.
2) Repeat the aspiration/wash as in step 2 of plate preparation.
3) Add 100 μL of the detection antibody, diluted in antibody dilution buffer, to each well. Seal the plate 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 50 μL 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 450 nm.

CALCULATION OF RESULTS

1. Calculate the mean absorbance for each set of duplicate standards, controls and samples. Subtract the mean zero standard absorbance from each.
2. 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.
3. 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.
4. Alternatively, computer-based curve-fitting statistical software may also be employed to calculate the concentration of the sample.

TYPICAL DATA

This standard curve is only for demonstration purposes. A standard curve should be generated for each assay.



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Concentration (pg/ml)	Zero standard subtracted OD
0	0.000
62.5	0.030
125	0.069
250	0.148
500	0.302
1000	0.602
2000	1.117
4000	2.028

ASSAY CHARACTERISTICS

SENSITIVITY

The minimum detectable dose of H1N1 (A/Puerto Rico/8/1934) HA was determined to be approximately 62.5 pg/ml. This is defined as at least three times standard deviations above the mean optical density of 10 replicates of the zero standard.

REFERENCES

1. Fouchier RA, Munster V, Wallensten A, et al. (March 2005). "Characterization of a novel influenza A virus hemagglutinin subtype (H16) obtained from black-headed gulls". J. Virol. 79 (5): 2814–22.
2. Suzuki Y (March 2005). "Sialobiology of influenza: molecular mechanism of host range variation of influenza viruses". Biol. Pharm. Bull. 28 (3): 399–408.
3. Gambaryan A, Tuzikov A, Pazynina G, Bovin N, Balish A, Klimov A (January 2006). "Evolution of the receptor binding phenotype of influenza A (H5) viruses". Virology 344 (2): 432–8.

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