2009 H1N1 Influenza (A/Brisbane/59/2007) Hemagglutinin ELISA Kit

Catalog Number: IT-E3Ag-2009H1N1

For the quantitative analysis of 2009 H1N1 influenza (A/Brisbane/59/2007) hemagglutinin concentrations in cell culture supernates and serum.

This package insert must be read in its entirety before using this product.

For Research Use Only. Not For Use In Diagnostic Procedures.

For research use only.
Not for use in Diagnostic procedures.

INTRODUCTION

Influenza (the flu) is a contagious respiratory illness caused by influenza viruses. The virus is categorized into three main types, namely A, B and C. Influenza A and B viruses that routinely spread in people are responsible for seasonal flu epidemics each year, causing upper and lower respiratory tract infection, and resulting a wide spectrum of mild to severe diseases including tracheobronchitis, viral pneumonia and encephalitis. Influenza type C infections cause a mild respiratory illness and are not thought to cause epidemics.

Influenza A viruses, found in a wide variety of mammalian and avian species, are divided into subtypes based on two proteins on the surface of the virus: the hemagglutinin (H) and the neuraminidase (N). There are 16 different hemagglutinin subtypes and 9 different neuraminidase subtypes. Influenza A viruses can be further broken down into different strains. While different combinations of the two antigens appear more frequently in some groups of birds than others, only few subtypes have established themselves in humans (HA: H1, H2, and H3; NA: N1and N2).

Influenza B is largely confined to humans and became unexpectedly prevalent in humans during 2000-2002. Although not divided into subtypes, influenza B viruses can be broken down into different strains.

Established in 1952, the WHO Influenza Surveillance Network serves as a global alert mechanism for the emergence of influenza viruses with epidemic and pandemic potential. It recommends twice annually the content of the influenza vaccine for the subsequent influenza season. For each season, three strains (H1N1, H3N2, and Type-B) are chosen in that year's flu vaccination. The chosen strains are thought most likely to cause significant human suffering in the coming season. The A/Brisbane/59/2007 is the chosen H1N1 strain in 2009.

The host restriction and virulence of an influenza virus is mainly determined by its HA protein, which binds to the monosaccharide sialic acid (SA) on the surface of its target cells. Human influenza HA preferentially binds to SA-α-2,6, while avian influenza HA preferentially binds to SA-α-2,3. To be infectious, HA must be cleaved into two subunits, HA1 and HA2, by proteases limited at respiratory tracts. However, the HA protein of highly pathogenic avian influenza virus strains contains a stretch of basic residues adjacent to the HA cleavage site, enabling its HA to be cleaved by proteases that are produced in many different tissues. As a result, these viruses can replicate in many organs, including the spleen, liver, lungs, kidneys, and brain, causing widespread disease and high mortality rates.

Immune Technology Corp. 954 Lexington Ave., #170, New York, NY 10021, USA
Phone: 1-866-448-8878 Email: info@immune-tech.com Web: www.immune-tech.com

PRINCIPLE OF THE ASSAY

This assay is based on the quantitative sandwich enzyme immunoassay technique, using a microplate pre-coated with a monoclonal antibody highly specific for 2009 H1N1 influenza (A/Brisbane/59/2007) HA. In short, standards and samples are added to the microplate. After HA is immobilized by the monoclonal antibody, the detection antibody is added to form a "sandwich" complex with the antigen. The detection antibody is a biotinylated polyclonal antibody specific for 2009 H1N1 influenza HA. After washes to remove any unbound polyclonal antibody, a horseradish peroxidase (HRP)-conjugated streptavidin is added. Following a wash to remove any unbound streptavidin-enzyme reagent, a substrate solution is added and color develops in proportion to the amount of 2009 H1N1 influenza (A/Brisbane/59/2007) HA retained on the plate. The color development is stopped and the intensity of the color is measured at 450 nm by a microplate reader.

LIMITATION OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- If samples generate values higher than the highest standard, further dilute the samples with binding buffer and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time and temperature, and kit age can cause variation in binding.
- Cell line tested did not have measureable levels of 2009 H1N1 influenza (A/Brisbane/59/2007) HA in this assay.
- This assay is designed to eliminate interference by binding proteins and other factor present in biological samples. Until all factors have been tested in this immunoassay, the possibility of interference can not be excluded.

Immune Technology Corp. 954 Lexington Ave., #170, New York, NY 10021, USA
Phone: 1-866-448-8878 Email: info@immune-tech.com Web: www.immune-tech.com

REAGENTS PROVIDED

1. **2009 H1N1 Influenza (A/Brisbane/59/2007) Hemagglutinin Monoclonal Antibody Coated Microtiter Plate (Part IT-E3Ag-2009H1N1-01):** 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against 2009 H1N1 influenza (A/Brisbane/59/2007) HA.
2. **H1N1 (A/Brisbane/59/2007) Detection Antibody (Part IT-E3Ag-2009H1N1-02):** 40 µL of polyclonal antibody against 2009 H1N1 influenza (A/Brisbane/59/2007) HA conjugated to biotin. Dilute to 11 mL with Assay Diluent before use.
3. **H1N1 (A/Brisbane/59/2007) Standard (Part IT-E3Ag-2009H1N1-03):** 40 µL of 8 µg/mL recombinant 2009 H1N1 influenza (A/Brisbane/59/2007) HA proetin.
4. **Streptavidin-HRP (Part IT-E-001):** 20 µL of HRP-conjugated Streptavidin. Dilute to 11 mL with Assay Diluent before use.
5. **Color Reagent (Part IT-E-002):** 12 mL of TMB peroxidase substrate, one component.
6. **Stop Solution (Part IT-E-003):** 12 mL of 1 N sulfuric acid.
7. **Assay Diluent Concentrate (Part IT-E-004):** 10 mL of 10-fold concentrated solution of a buffered protein base with preservative.
8. **Wash Buffer Concentrate (Part IT-E-005):** 50 mL of 10-fold concentrated solution of surfactant with preservative.

OTHER SUPPLIES REQUIRED

- Microtiter Plate Reader (450 nm).
- Microtiter Plate Washer.
- Pipettes, multi-channel pipettes and pipette tips.
- Deionized or distilled water.
- Polypropylene reagent tubes.

PRECAUTION

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

STORAGE

Keep the microplate and all reagents in a kit at 4^{o}C, and protect Streptavidin-HRP from prolonged exposure to light.

Immune Technology Corp. 954 Lexington Ave., #170, New York, NY 10021, USA
Phone: 1-866-448-8878 Email: info@immune-tech.com Web: www.immune-tech.com

SAMPLE COLLECTION AND STORAGE

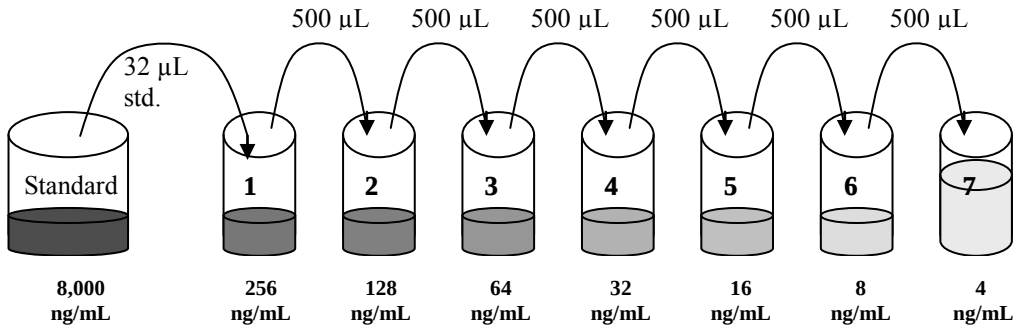
Warning: Polypropylene tubes must be used. Do not use glass.

- **Cell Culture Supernates:** Remove particulates by centrifugation at 1000 x g and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.
- **Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.
- **Avoid adding sodium azide to the sample, since it will inactivate the horseradish peroxidase (HRP) enzyme activity.**

REAGENT PREPARATION

Bring all reagents to room temperature before use.

- **Assay Diluent:** Dilute 10 mL of Assay Diluent Concentrate into deionized or distilled water to prepare 100 mL of Assay Diluent.
- **Wash Buffer:** Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 500 mL of Wash Buffer.
- **Standard Dilutions:** Pipette 968 µL of Assay Diluent into the #1 tube. Pipette 500 µL of Assay Diluent into the remaining tubes. Use the stock solution to produces a dilution series (see below). Mix each tube thoroughly before the next transfer. The tube #1 serves as the highest standard. Assay diluent serves as the zero standard (0pg/mL).



Immune Technology Corp. 954 Lexington Ave., #170, New York, NY 10021, USA
Phone: 1-866-448-8878 Email: info@immune-tech.com Web: www.immune-tech.com

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples and standard be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous section.
2. Remove excess microplate strips from the plate frame, return them to the zip bag containing the desiccant pack, and reseal tightly.
3. Add 100 µL of Standard Dilution or sample per well. Incubate for 2 hours at room temperature. (See an ELISA plate template on Page 8).
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 300 µL of Wash Buffer. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100 µL of the detection antibody, diluted in Assay Diluent, to each well. Incubate 1 hour at room temperature.
6. Repeat the aspiration/wash step as in step 4.
7. Add 100 µL of the Streptavidin-HRP, diluted in Assay Diluent, to each well. Incubate 30 minutes at room temperature. **Protect from light.**
8. Repeat the aspiration/wash step as in step 4.
9. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
10. Add 100 µL of Stop Solution to each well. If color change does not appear uniform, gently tap plate to ensure thorough mixing.
11. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm.

Immune Technology Corp. 954 Lexington Ave., #170, New York, NY 10021, USA
Phone: 1-866-448-8878 Email: info@immune-tech.com Web: www.immune-tech.com

CALCULATION OF RESULTS

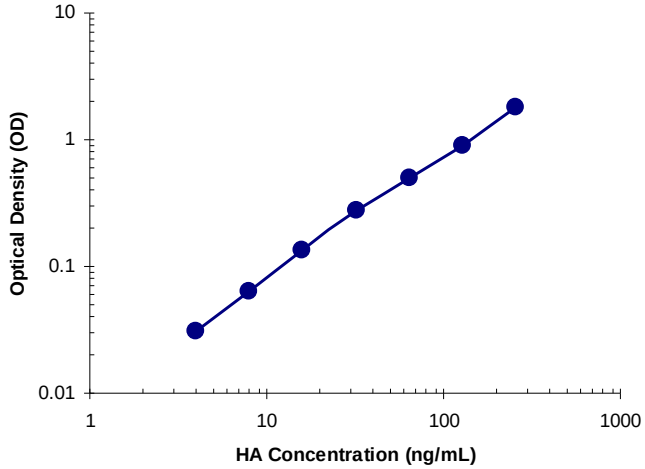
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, 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. The data may be linearized by plotting the log of the HA concentrations versus the log of the O.D., and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



Concentration (ng/mL)	Zero Standard Subtracted OD
0	0
4	0.031
8	0.064
16	0.1325
32	0.2735
64	0.4975
128	0.9065
256	1.811

Immune Technology Corp. 954 Lexington Ave., #170, New York, NY 10021, USA
Phone: 1-866-448-8878 Email: info@immune-tech.com Web: www.immune-tech.com

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

SENSITIVITY

The minimum detectable dose of 2009 H1N1 influenza (A/Brisbane/59/2007) hemagglutinin was to be approximately 4 ng/mL. This was determined by adding two standard deviation to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against highly purified, HEK293-expressed, recombinant 2009 H1N1 influenza (A/Brisbane/59/2007) HA produced at Immune Technology Corp.

SPECIFICITY

The following hemagglutinin of different influenza virus types and subtypes prepared at 200 ng/mL were tested and no cross-reactivity was identified:

H1N1 (Swine Flu)
H5N1(A/chicken/Vietnam/NCVD-016/08(H5N1))
Influenza B (B/Brisbane/60/2008)
H3N2 (A/Brisbane/10/2007)

Immune Technology Corp. 954 Lexington Ave., #170, New York, NY 10021, USA
Phone: 1-866-448-8878 Email: info@immune-tech.com Web: www.immune-tech.com

ELISA Plate Template

	1	2	3	4	5	6	7	8	9	10	11	12
A	Tube #1, std	Tube #1, std										
B	Tube #2, std	Tube #2, std										
C	Tube #3, std	Tube #3, std										
D	Tube #4, std	Tube #4, std										
E	Tube #5, std	Tube #5, std										
F	Tube #6, std	Tube #6, std										
G	Tube #7, std	Tube #7, std										
H	Assay Diluent	Assay Diluent										

Immune Technology Corp. 954 Lexington Ave., #170, New York, NY 10021, USA
Phone: 1-866-448-8878 Email: info@immune-tech.com Web: www.immune-tech.com