



Sino Biological
Biological Solution Specialist

Influenza A H1N1 (Swine Flu 2009) Hemagglutinin / HA ELISA Pair Set

Catalog Number : SEK001
Lot Nmuber: KW17DE2901

To achieve the best assay results, this manual must be read carefully before using this product and the assay is run as summarized in the General ELISA protocol.

BACKGROUND

The influenza viral Hemagglutinin (HA) protein is a homotrimer with a receptor binding pocket on the globular head of each monomer. HA has at least 18 different antigens. These subtypes are named H1 through H18. HA has two functions. Firstly, it allows the recognition of target vertebrate cells, accomplished through the binding to these cells' sialic acid-containing receptors. Secondly, once bound it facilitates the entry of the viral genome into the target cells by causing the fusion of the host endosomal membrane with the viral membrane. The influenza virus Hemagglutinin (HA) protein is translated in cells as a single protein, HA, or hemagglutinin precursor protein. For viral activation, hemagglutinin precursor protein (HA) must be cleaved by a trypsin-like serine endoprotease at a specific site, normally coded for by a single basic amino acid (usually arginine) between the HA1 and HA2 domains of the protein. After cleavage, the two disulfide-bonded protein domains produce the mature form of the protein subunits as a prerequisite for the conformational change necessary for fusion and hence viral infectivity.

PRINCIPLE OF THE TEST

The Sino Biological ELISA Pair Set is a solid phase sandwich ELISA (Enzyme-Linked Immunosorbent Assay). It utilizes a monoclonal antibody specific for Influenza A H1N1 (Swine Flu 2009) Hemagglutinin / HA coated on a 96-well plate. Standards and samples are added to the wells, and any Influenza A H1N1 (Swine Flu 2009) Hemagglutinin / HA present binds to the immobilized antibody. The wells are washed and a horseradish peroxidase conjugated rabbit anti-Influenza A H1N1 (Swine Flu 2009) Hemagglutinin / HA polyclonal 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 Influenza A H1N1 (Swine Flu 2009) Hemagglutinin / 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.

INTENDED USE

- ◆ The Influenza A H1N1 (Swine Flu 2009) Hemagglutinin / HA ELISA Pair Set is for the quantitative determination of Influenza A H1N1 (Swine Flu 2009) Hemagglutinin / HA.
- ◆ This ELISA Pair Set contains the basic components required for the development of sandwich ELISAs.

ASSAY PROCEDURE SUMMARY



This Pair Set has been configured for research use only and is not to be used in diagnostic procedures.

MATERIALS PROVIDED

Bring all reagents to room temperature before use.

Capture Antibody – 1 mg/mL of mouse anti-Influenza A H1N1 (Swine Flu 2009) Hemagglutinin / HA monoclonal antibody (in PBS, pH 7.4). Dilute to a working concentration of 2 µg/mL in PBS before coating. (Catalog: # CTA01)

Detection Antibody – 0.2 mg/mL of rabbit anti-Influenza A H1N1 (Swine Flu 2009) Hemagglutinin / HA polyclonal antibody conjugated to horseradish-peroxidase (HRP) (in PBS, 50 % HRP-Protector, pH 7.4, store at 4°C). Dilute to working concentration of 1 µg/mL in dilution buffer before use.

Standard – Each vial contains 77 ng of recombinant Influenza A H1N1 (Swine Flu 2009) Hemagglutinin / HA. Reconstitute with 1 mL dilution buffer. After reconstitution, store at -20°C to -80°C in a manual defrost freezer. A seven-point standard curve using 2-fold serial dilutions in dilution buffer, and a high standard of 2000 pg/mL is recommended.

Standard reconstitution tips: Add dilution buffer, gently mix it up and down 3~5 times. Avoid violent and long-time shock.

SOLUTIONS REQUIRED

PBS - 136.9 mM NaCl, 10.1 mM Na₂HPO₄, 2.7 mM KCl, 1.8 mM KH₂PO₄, pH 7.4, 0.2 µm filtered

Wash Buffer - 0.05% Tween20 in PBS, pH 7.2 - 7.4

Blocking Buffer - 2% BSA in Wash Buffer

Dilution Buffer - 0.1% BSA in wash buffer, pH 7.2 - 7.4, 0.2 µm filtered

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

Substrate stock solution - 10mg / ml TMB (Tetramethylbenzidine) 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 80 µl 0.75% H₂O₂ , mix it well

Stop Solution - 2 N H₂SO₄

PRECAUTION

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

STORAGE

Capture Antibody: Aliquot and store at -20°C to -80°C for up to 6 months from date of receipt. Avoid repeated freeze-thaw cycles.

Detection Antibody: Store at 4°C and protect it from prolonged exposure to light for up to 6 months from date of receipt. **DO NOT FREEZE!**

Standard: Store lyophilized standard at -20°C to -80°C for up to 6 months from date of receipt. Aliquot and store the reconstituted standard at -80°C for up to 1 month. Avoid repeated freeze-thaw cycles.

GENERAL ELISA PROTOCOL

Plate Preparation

1. Dilute the capture antibody to the working concentration in PBS. Immediately coat a 96-well microplate with 100 μ L 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 300 μ L 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 μ L 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.

Assay Procedure

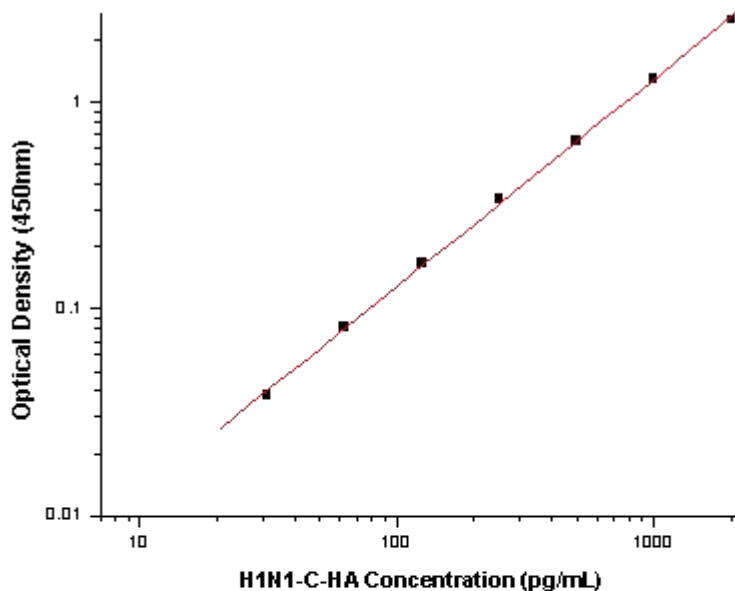
1. Add 100 μ L of sample or standards in Dilution Buffer per well. Seal the plate and incubate 2 hours at room temperature.
2. Repeat the aspiration/wash as in step 2 of plate preparation.
3. Add 100 μ L of the detection antibody, diluted in Dilution Buffer, to each well. Seal the plate and incubate 1 hour at room temperature.
4. Repeat the aspiration/wash as in step 2 of plate preparation.
5. Add 200 μ L 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 μ L 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

- 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.

TYPICAL DATA

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



Concentration (pg/mL)	Zero standard subtracted OD
0	0
31.25	0.038
62.50	0.082
125.00	0.167
250	0.340
500	0.654
1000	1.291
2000	2.528

PERFORMANCE CHARACTERISTIC

SENSITIVITY

The minimum detectable dose of Influenza A H1N1 (Swine Flu 2009) Hemagglutinin / HA was determined to be approximately 31.25 pg/ml. This is defined as at least three times standard deviations above the mean optical density of 10 replicates of the zero standard.

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 (A/Brisbane/59/2007)

H5N1 (A/Anhui/1/2005)

H5N1 (A/Vietnam/1194/2004)

Influenza B (B/Florida/4/2006)

H3N2 (A/Brisbane/10/2007)

TROUBLE SHOOTING

Problems	Possible Sources	Solutions
No signal	Incorrect or no Detection Antibody was added	Add appropriate Detection Antibody and continue
	Substrate solution was not added	Add substrate solution and continue
	Incorrect storage condition	Check if the kit is stored at recommended condition and used before expiration date
Poor Standard Curve	Standard was incompletely reconstituted or was inappropriately stored	Aliquot reconstituted standard and store at -80 °C
	Imprecise / inaccurate pipetting	Check / calibrate pipettes
	Incubations done at inappropriate temperature, timing or agitation	Follow the general ELISA protocol
	Background wells were contaminated	Avoid cross contamination by using the sealer appropriately
Poor detection value	The concentration of antigen in samples was too low	Enriching samples to increase the concentration of antigen
	Samples were ineffective	Check if the samples are stored at cold environment. Detect samples in timely manner
High Background	Insufficient washes	Use multichannel pipettes without touching the reagents on the plate
		Increase cycles of washes and soaking time between washes
	TMB Substrate Solution was contaminated	TMB Substrate Solution should be clear and colorless prior to addition to wells
	Materials were contaminated.	Use clean plates, tubes and pipettes tips
Non-specificity	Samples were contaminated	Avoid cross contamination of samples
	The concentration of samples was too high	Try higher dilution rate of samples

ELISA Plate Template												
	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

**Influenza A H1N1 (Swine Flu 2009) Hemagglutinin / HA ELISA
Pair Set
Notes**