H5N1 (Avian Flu) Hemagglutinin ELISA Pair Set

Catalog Number : SEK002

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.

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BACKGROUND

Influenza (flu) is a viral 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 mammalian and avian species and is associated with the major human pandemics. Influenza B is largely confined to humans and became unexpectedly prevalent in humans during 2000-2002. Influenza C infects humans, dogs and pigs and generally causes only mild upper respiratory tract infection. However, influenza A and B viruses cause a wide spectrum of severe disease including lower respiratory, tract infection, pneumonia and encephalitis. Influenza A is further divided into subtypes based on antigenic differences in the membrane proteins hemagglutinin (HA) and neuraminidase (NA). 16 HAs (H1-H16) and 9 NA (N1-N9) had been identified. 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: N1 and N2).

Sustained, widespread highly pathogenic avian influenza (HPAI) H5N1 epidemics represent a significant public health hazard because they not only cause mortality in poultry but also increase the risk of a human influenza pandemic. In 1997, the first case of human infected H5N1 was reported in Hongkong, China. Avian influenza have broken the species barrier and made heavy threaten to human health. In the process of epidemic, more than 100 countries had found more than 400 human avian influenza infected cases, within 262 patients dead. Since two human-to-human transmission cases were reported in China, controlling the interspecies transmission of avian influenza is of critical importance.

At least 4 major antigenic groups of H5N1 viruses currently in circulation have caused infection in humans. The recent emergence and development of the unique antigenic FJ-like virus (clade 2.3.4) in Asia, the continuous circulation and expansion of Qinghai-like virus (clade 2.2) in Europe, the Middle East, and Africa, and the persistent prevalence of clade 2.1 viruses in Indonesia, and classic Clade 1 in Vietnam and Hong Kong.

Hemagglutinin (HA), which binds to sialic acid (SA)-containing receptors on host cells, is the protein that produces neutralizing antibodies. Hemagglutinin plays a major role in the determination of host range restriction and virulence because human influenza HA preferentially binds to SA-α-2,6 while avian influenza HA preferentially binds to SA-α-2,3. The cleavage of HA into two disulfide-linked subunits, HA1 and HA2, is a prerequisite for initiating infection. Usually HA is restricted to be cleaved at respiratory tracts by limited proteases. Highly pathogenic avian influenza contains a stretch of basic residues adjacent to the HA cleavage site, enabling its HA to be cleaved by a wide range of proteases with ubiquitous tissue distributions. This process permits productive virus replication in organs outside of the respiratory and gastrointestinal tracts, including the brain, resulting in widespread disease and high mortality rates.

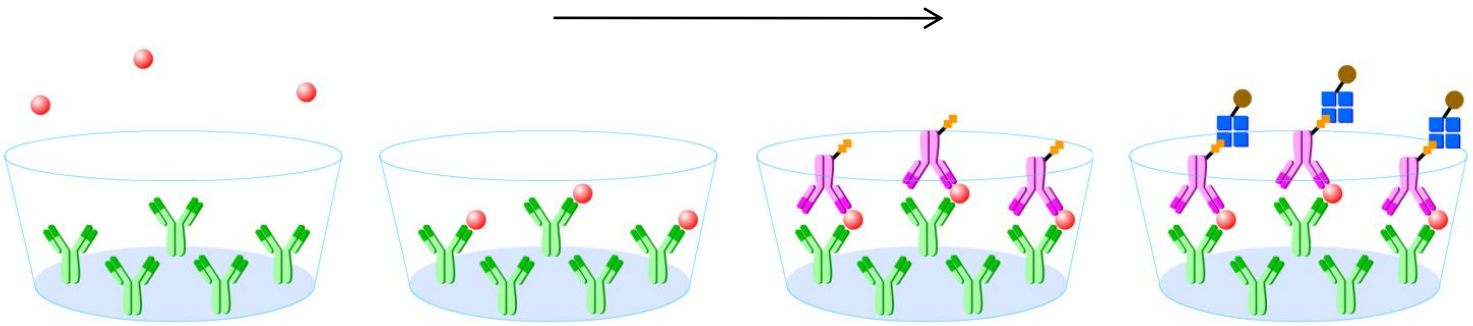
PRINCIPLE OF THE TEST

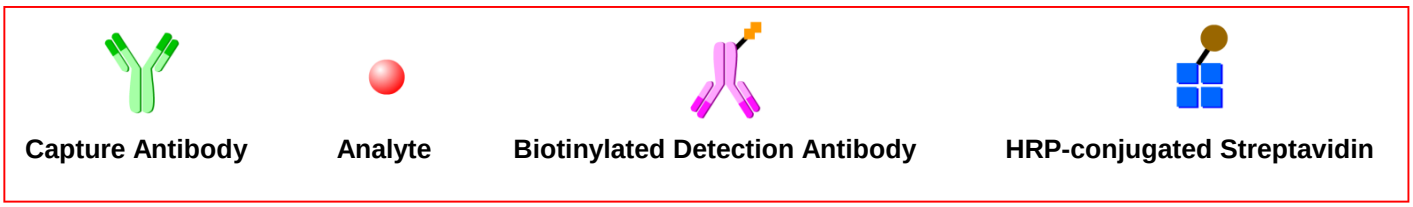
The Sino Biological ELISA Pair Set is a solid phase sandwich ELISA (Enzyme-Linked Immunosorbent Assay). It utilizes a monoclonal antibody specific for hemagglutinin coated on a 96-well plate. Standards and samples are added to the wells, and any hemagglutinin present binds to the immobilized antibody. The wells are washed and a biotinylated rabbit anti-hemagglutinin polyclonal antibody is then added, producing an antibody-antigen-antibody “sandwich”. To produces color in proportion to the amount of hemagglutinin present in the sample strepavidin-HRP and TMB substrate solution are loaded. The absorbances of the microwell are read at 450 nm.

INTENDED USE

- The H5N1 (avian flu) hemagglutinin ELISA Pair Set is for the quantitative determination of hemagglutinin.
- 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.

SPECIFICITY

This H5N1 HA / Hemagglutinin ELISA Pair Set (SEK002) can quantificationally detect Anhui-HA (A/Anhui/1/2005), Indonesia-HA (A/Indonesia/5/2005), and Vietnam-HA (A/Vietnam/1194/2004).

And it can also react with Turkey-HA (A/turkey/Turkey/1/2005) and Qinghai-HA (A/bar-headed goose/Qinghai/14/2008).

MATERIALS PROVIDED

Bring all reagents to room temperature before use.

Capture Antibody – 0.5 mg/mL of mouse anti-H5N1 hemagglutinin monoclonal antibody. Dilute to a working concentration of 0.5 µg/mL in PBS before coating. (Catalog: # 11048-MM14)

Detection Antibody - 0.5 mg/mL biotinylated rabbit anti-H5N1 hemagglutinin polyclonal antibody. Dilute to a working concentration of 1.5 µg/mL in detection antibody dilution buffer before use.

Standard - Each vial contains 150 ng of recombinant H5N1 hemagglutinin. Reconstitute with 1 mL detection antibody dilution buffer. A seven-point standard curve usi ng 2-fold serial dilutions in sample dilution buffer, and a high standard of 5000 pg/mL is recommended.

Streptavidin-HRP - 50 µL of streptavidin conjugated to horseradish-peroxidase. 1:2000 Dilution in detection antibody dilution buffer before use.

SOLUTIONS REQUIRED

PBS - 136.9 mM NaCl, 10.1 mM Na_2HPO_4, 2.7 mM KCl, 1.8 mM KH_2PO_4, pH 7.4, 0.2 µm filtered

TBS - 20mM Tris , 150 mM NaCl , pH 7.4

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, pH 7.2 - 7.4, 0.2 µm filtered

Detection antibody dilution buffer - 0.5% 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 - 10 mg/ml TMB (Tetramethylbenzidine) in DMSO

Substrate dilution buffer - 0.05M Na_2HPO_4 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% H_2O_2 , mix it well

Stop Solution - 2 N H_2SO_4

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℃ to -80℃ for up to 6 months from date of receipt. Avoid repeated freeze-thaw cycles.

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

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

Streptavidin-HRP: Store at 4℃ and protect it from prolonged exposure to light. **DO NOT FREEZE!** It is stable for up to 6 months from date of receipt.

GENERAL ELISA PROTOCOL

Plate Preparation

1. Dilute the capture antibody to the working concentration in PBS. 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.

Assay Procedure

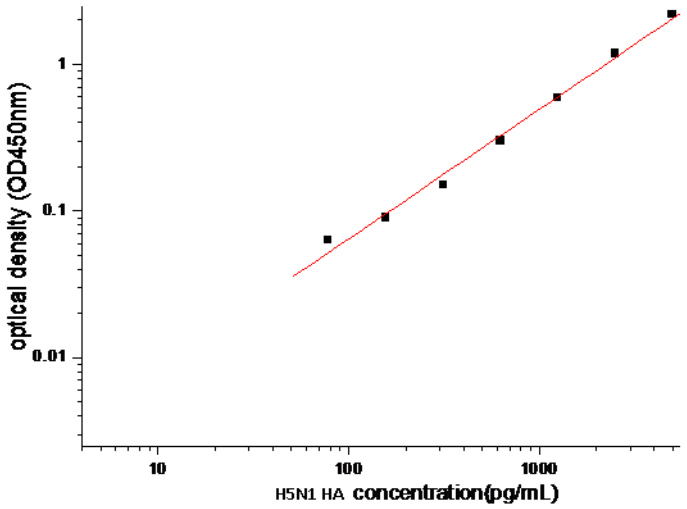
1. Add 100 μL of sample or standards in sample dilution buffer per well. Seal the plate and incubate 2 hours at room temperature.
2. Repeat the aspiration/wash as in step 2 of plate preparation.
3. Add 100 μL of the detection antibody, diluted in antibody dilution buffer, to each well. Seal the plate and incubate 1 hour at room temperature.
4. Repeat the aspiration/wash as in step 2 of plate preparation.
5. Add 100 μL of Streptavidin-HRP to each well. Incubate for 1 hour at room temperature.
6. Repeat the aspiration/wash as in step 2 of plate preparation.
7. 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.
8. Add 50 μL of stop solution to each well. Gently tap the plate to ensure thorough mixing.
9. 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	0.000
78.125	0.063
156.25	0.090
312.5	0.150
625	0.300
1250	0.592
2500	1.177
5000	2.191

PERFORMANCE CHARACTERISTIC

SENSITIVITY

The minimum detectable dose of H5N1 hemagglutinin (HA) was determined to be approximately **78.125 pg/ml**. This is defined as at least three times standard deviations above the mean optical density of 10 replicates of the zero standard.

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 ℃
	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												

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Notes