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User's Manual

Influenza A H5N1 (Avian Flu) Hemagglutinin / HA ELISA Pair Set

REF **ABPR-ZB322**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The H5N1 (avian flu) hemagglutinin ELISA Pair Set is for the quantitative determination of H5N1 (avian flu) hemagglutinin. This ELISA Pair Set contains the basic components required for the development of sandwich ELISAs.

Principles of Testing

The ELISA Pair Set is a solid phase sandwich ELISA (Enzyme-Linked Immunosorbent Assay). It utilizes a monoclonal antibody specific for H5N1 (avian flu) hemagglutinin coated on a 96-well plate. Standards and samples are added to the wells, and any H5N1 (avian flu) hemagglutinin present binds to the immobilized antibody. The wells are washed and a horseradish peroxidase conjugated mouse anti-H5N1 (avian flu) hemagglutinin 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 H5N1 (avian flu) hemagglutinin 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 And Materials Provided

Bring all reagents to room temperature before use.

Capture Antibody: 1 mg/mL of mouse anti-H5N1 hemagglutinin monoclonal antibody (in PBS, pH 7.4). Dilute to a working concentration of 2 µg/mL in PBS before coating.

Detection Antibody: 0.2 mg/mL of rabbit anti-Influenza A H5N1 hemagglutinin polyclonal antibody conjugated to horseradish-peroxidase (HRP) (in PBS, 50 % HRP-Protector, pH 7.4, store at 4°C). Dilute to working concentration of 0.7 µg/mL in dilution buffer before use.

Standard: Each vial contains 299 ng of recombinant H5N1 hemagglutinin. Reconstitute with 1 mL dilution buffer. A seven-point standard curve usi ng 2-fold serial dilutions in dilution buffer, and a high standard of 8000 pg/mL is recommended.

Materials Required But Not Supplied

PBS: 136.9 mM NaCl, 10.1 mM Na_2HPO_4, 2.7 mM KCl, 1.8 mM KH_2PO_4, pH 7.4, 0.2µm 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 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 80 µl 0.75% H_2O_2, mix it well

Stop Solution: 2 N H_2SO_4

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 freezethaw cycles.

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°C.
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 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 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

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

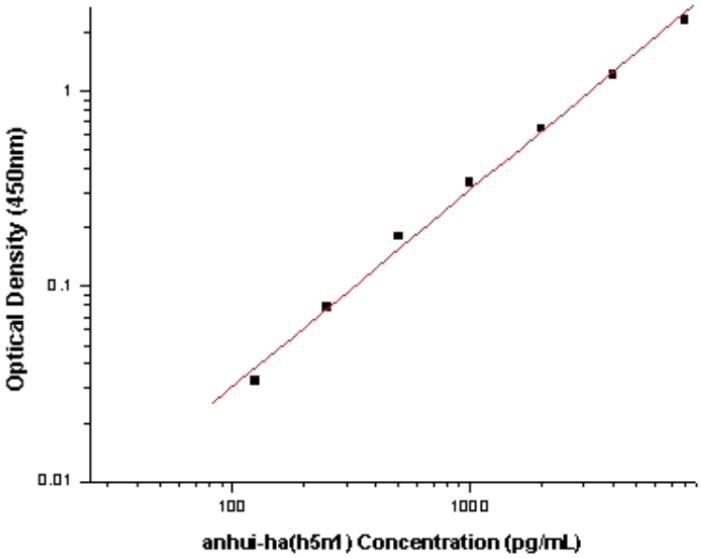
Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.

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 Standard Curve

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
125	0.033
250	0.078
500	0.181
1000	0.339
2000	0.648
4000	1.228
8000	2.335

Detection Range

125-8000 pg/mL

Sensitivity

The minimum detectable dose of H5N1 hemagglutinin (HA) was determined to be approximately 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.