Product Manual

Influenza A Nucleoprotein ELISA Kit

Catalog Numbers

VPK-5174	96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

The influenza virus is an enveloped virus that can be divided into three classes, A, B, and C, largely based upon conserved antigenic differences in the internal nucleoprotein (NP). Only Influenza A and B are clinically relevant for humans. Influenza A virus, typically encountered more frequently than type B, is associated with the majority of serious epidemics, and it can be further subdivided into strains or subtypes based on antigenic differences in the external hemagglutinin proteins (H1-H16) and neuraminidase proteins (N1-N9).

The primary function of NP is to encapsidate the segmented RNA and bind with the three polymerase subunits, PA, PB1 and PB2, to form ribonucleoprotein particles (RNPs) for RNA transcription, replication and packaging.

Cell Biolabs' Influenza A nucleoprotein ELISA Kit is an enzyme immunoassay developed for detection and quantitation of the Influenza A nucleoprotein. The ELISA antibodies only recognize the nucleoprotein from Influenza A, and will not react with the nucleoprotein from Influenza B nor Influenza C. The kit has a detection sensitivity limit of 625 pg/mL Influenza A nucleoprotein. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and Influenza A lysate samples.

Assay Principle

An anti-Influenza A nucleoprotein monoclonal antibody is adsorbed onto a microtiter plate. Influenza A nucleoprotein present in the sample or standard binds to the antibody adsorbed on the plate; an FITC-conjugated anti-Influenza A nucleoprotein monoclonal antibody is added and binds to the Influenza A nucleoprotein captured by the first antibody. Following incubation and wash steps, an HRP-conjugated mouse anti-FITC antibody is added and binds to the FITC conjugated anti-Influenza A nucleoprotein monoclonal antibody. Unbound HRP-conjugated mouse anti-FITC antibody is removed during a wash step, and a substrate solution reactive with HRP is added to the wells. A colored product is formed in proportion to the amount of Influenza A nucleoprotein present in the sample. The reaction is terminated by addition of Stop Solution and absorbance is measured at 450 nm. A standard curve is prepared from the provided recombinant Influenza A nucleoprotein standard, and the sample Influenza A nucleoprotein concentration is then determined.

Related Products

1. VPK-108-H: QuickTiter™ Lentivirus Quantitation Kit (HIV-1 p24 ELISA)
2. VPK-150: QuickTiter™ Hepatitis B Core Antigen (HBcAg) ELISA Kit
3. VPK-151: QuickTiter™ Hepatitis C Core Antigen (HCcAg) ELISA Kit
4. VPK-156: QuickTiter™ MuLV Core Antigen (MuLV p30) ELISA Kit
5. VPK-5169: Adenovirus Hexon ELISA Kit
6. VPK-5170: RSV Fusion Protein ELISA Kit
7. VPK-5171: RSV Nucleoprotein ELISA Kit
8. VPK-5175: Influenza B Nucleoprotein ELISA Kit

Kit Components

Box 1 (shipped at room temperature)

1. Anti-Influenza A Nucleoprotein Antibody Coated Plate (Part No. 51741B): One strip well 96-well plate.
2. FITC-Conjugated Anti-Influenza A Nucleoprotein Monoclonal Antibody (Part No. 51742C): One 20 µL vial.
3. HRP-Conjugated Anti-FITC Monoclonal Antibody (Part No. 310811): One 20 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Viral Lysis Buffer (Part No. 51693B): One 15 mL bottle containing 200 mM Tris, pH 7.5, 1500 mM NaCl, 10% Triton X-100, 1% SDS.
6. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
7. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
8. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. Recombinant Influenza A Nucleoprotein Standard (Part No. 51743D): One 100 µL vial of 4 µg/mL recombinant human Influenza B nucleoprotein (Met1-Tyr560) in PBS containing BSA.

Materials Not Supplied

1. Influenza A Sample: purified virus or unpurified viral supernatant
2. Microcentrifuge
3. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
4. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
5. Multichannel micropipette reservoir
6. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receiving, aliquot and store the Influenza A Nucleoprotein Standard at -20ºC and avoid freeze/thaw. Store all other components at 4ºC.

Safety Considerations

Remember that your Influenza A samples contain infectious viruses before inactivation; you must follow the recommended NIH guidelines for all materials containing infectious organisms.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity.
- FITC-Conjugated Anti-Influenza A Nucleoprotein Monoclonal Antibody and HRP-Conjugated Anti-FITC Monoclonal Antibody: Immediately before use dilute the FITC-conjugated antibody 1:1000 and HRP-conjugated antibody 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

1. Prepare a dilution series of Influenza A Nucleoprotein Standard in the concentration range of 40 ng/mL – 0.625 ng/mL by diluting the stock solution in Assay Diluent (Table 1).

Standard Tubes	4 µg/mL Influenza A Nucleoprotein Standard (µL)	Assay Diluent (µL)	Influenza A Nucleoprotein (ng/mL)
1	10	990	40
2	500 of Tube #1	500	20
3	500 of Tube #2	500	10
4	500 of Tube #3	500	5
5	500 of Tube #4	500	2.5
6	500 of Tube #5	500	1.25
7	500 of Tube #6	500	0.625
8	0	500	0

Table 1. Preparation of Influenza B Nucleoprotein Standard

2. Transfer 225 µL of each dilution to a microcentrifuge tube containing 25 µL of 10X Lysis Buffer. Perform the assay as described in Assay Protocol.

Influenza Virus Sample Inactivation and Lysis

1. (Optional) Dilute Influenza A samples in culture medium. Include culture medium as a negative control.
2. Transfer 225 µL of each sample to a microcentrifuge tube containing 25 µL of 10X Lysis Buffer, vortex well. Inactivate Influenza A sample at 56ºC for 30 min.
3. Centrifuge at 12,000 x g for 5 minutes at 4°C. Collect the supernatant as Influenza A lysate.

Assay Protocol

1. Prepare and mix all reagents thoroughly before use.
2. Each Influenza A lysate sample, Influenza A nucleoprotein standard, blank, and control medium should be assayed in duplicate.

3. Add 100 µL of Influenza A lysate or Influenza A nucleoprotein standard to Anti-Influenza A Nucleoprotein Antibody Coated Plate.
4. Cover with a Plate Cover and incubate at 37ºC for 2 hours.
5. Remove plate cover and empty wells. Wash microwell strips 5 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
6. Add 100 µL of the diluted FITC-Conjugated Anti-Influenza A Nucleoprotein Monoclonal Antibody to each well.
7. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.
8. Remove plate cover and empty wells. Wash the strip wells 5 times according to step 5 above.
9. Add 100 µL of the diluted HRP-Conjugated Anti-FITC Monoclonal Antibody to all wells.
10. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.
11. Remove plate cover and empty wells. Wash microwell strips 5 times according to step 5 above. Proceed immediately to the next step.
12. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes.

    Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.

13. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
14. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical Influenza A Nucleoprotein ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.

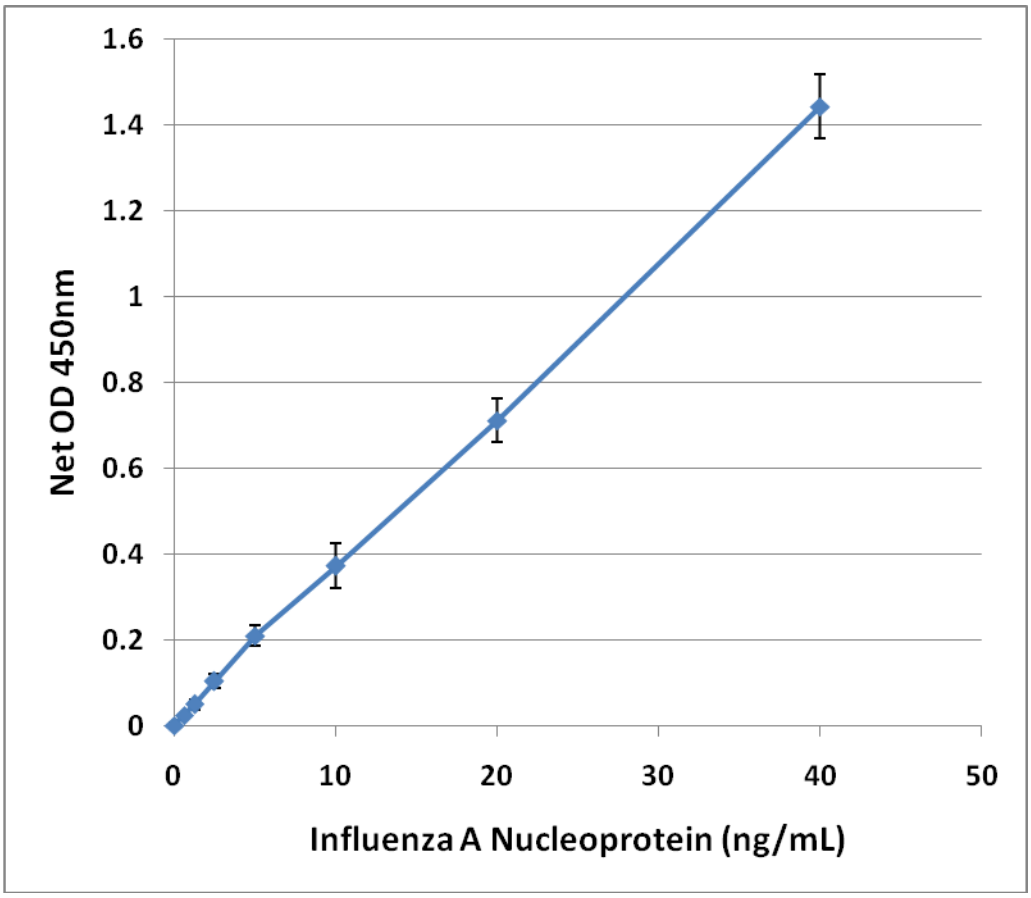

Figure 1: Influenza A Nucleoprotein ELISA Standard Curve.

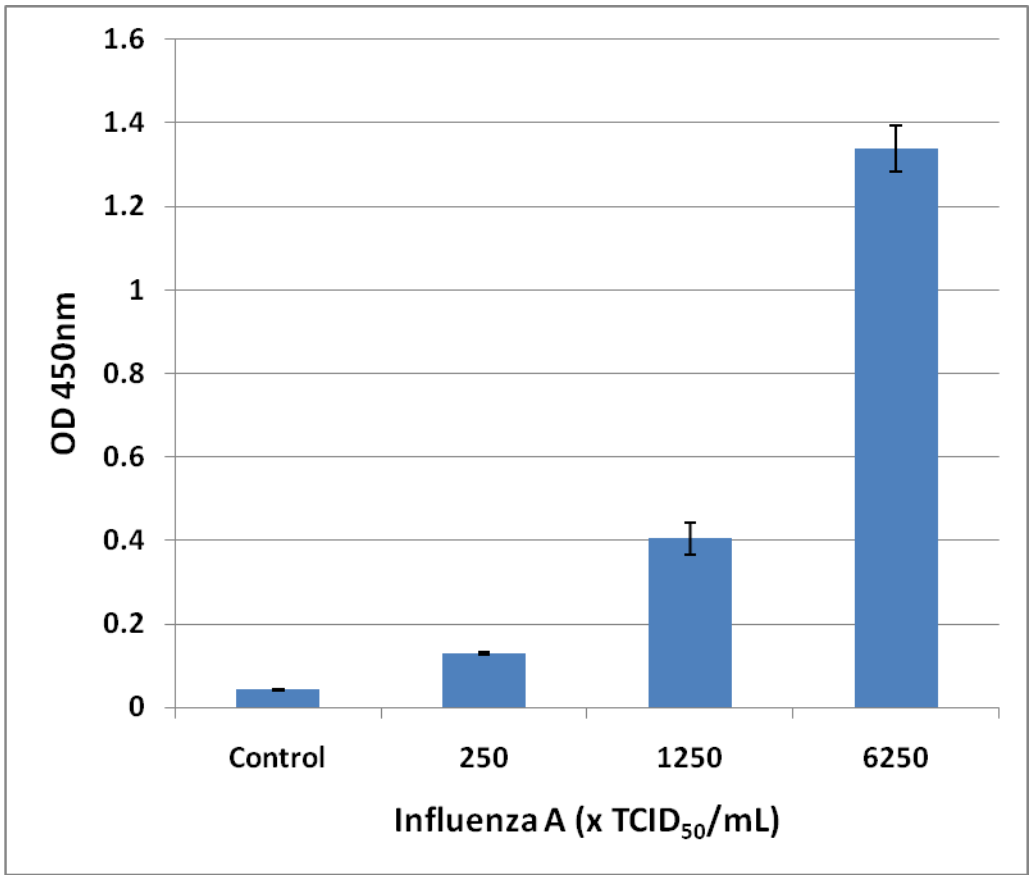

Figure 2: Influenza A Nucleoprotein in Influenza A Culture Fluid. Influenza A culture fluid (3.16 x 10^6 $TCID_{50}$/mL) was first diluted 100-fold with culture medium, then heat inactivated and lysed in Viral Lysis Buffer. Influenza A lysate was subjected to Influenza A Nucleoprotein ELISA Kit according to Assay Protocol.

References

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