Version 27-12/24

User's Manual

Influenza B Virus Nucleoprotein Antigen ELISA Kit

REF **DEIAFY108**

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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.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

The Influenza B Nucleoprotein ELISA Kit is an enzyme immunoassay developed for detection and quantitation of the Influenza B nucleoprotein. The ELISA antibodies only recognize the nucleoprotein from Influenza B, will not react with the nucleoprotein from Influenza A nor Influenza C. The kit has a detection sensitivity limit of 313 pg /mL Influenza B nucleoprotein. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and Influenza B lysate samples.

General Description

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.

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.

Principles of Testing

An anti-Influenza B nucleoprotein monoclonal antibody is adsorbed onto a microtiter plate. Influenza B nucleoprotein present in the sample or standard binds to the antibody adsorbed on the plate; an FITC-conjugated anti-Influenza B nucleoprotein monoclonal antibody is added and binds to the Influenza B 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 B 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 B 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 B nucleoprotein standard, and sample Influenza B nucleoprotein concentration is then determined.

Reagents And Materials Provided

Box 1 (shipped at room temperature)

1. Anti-Influenza B Nucleoprotein Antibody Coated Plate: One strip well 96-well plate.
2. FITC-Conjugated Anti-Influenza B Nucleoprotein Monoclonal Antibody: One 20 µL vial.
3. HRP-Conjugated Anti-FITC Monoclonal Antibody: One 20 µL vial.
4. Assay Diluent: One 50 mL bottle.
5. 10× Viral Lysis Buffer: One 15 mL bottle containing 200 mM Tris, pH 7.5, 1500 mM NaCl, 10% Triton X-100, 1% SDS.
6. 10× Wash Buffer: One 100 mL bottle.

7. Substrate Solution: One 12 mL amber bottle.
8. Stop Solution: One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. Recombinant Influenza B Nucleoprotein Standard: One 100 µL vial of 2 µg/mL recombinant human Influenza B nucleoprotein (Met1-Tyr560) in PBS containing BSA.

Materials Required But Not Supplied

1. Influenza B 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 -20°C and avoid freeze/thaw. Store all other components at 4°C.

Specimen Collection And Preparation

Influenza Virus Sample Inactivation and Lysis

1. (Optional) Dilute Influenza B 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 10× Lysis Buffer, vortex well. Inactivate Influenza B sample at 56°C for 30 min.
3. Centrifuge at 12,000 x g for 5 minutes at 4°C. Collect the supernatant as Influenza B lysate.

Reagent Preparation

1. 1× Wash Buffer: Dilute the 10× Wash Buffer Concentrate to 1× with deionized water. Stir to homogeneity.
2. FITC-Conjugated Anti-Influenza B 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.
3. Preparation of Standard Curve

a. Prepare a dilution series of Influenza B Nucleoprotein Standard in the concentration range of 20 ng/mL – 0.313 ng/mL by diluting the stock solution in Assay Diluent (Table 1).

Standard Tubes	2 μg/mL Influenza B Nucleoprotein Standard (μL)	Assay Diluent (μL)	Influenza B Nucleoprotein (ng/mL)
1	10	990	20
2	500 of Tube #1	500	10
3	500 of Tube #2	500	5
4	500 of Tube #3	500	2.5
5	500 of Tube #4	500	1.25
6	500 of Tube #5	500	0.625
7	500 of Tube #6	500	0.313
8	0	500	0

Table 1. Preparation of Influenza B Nucleoprotein Standard

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

Assay Procedure

1. Prepare and mix all reagents thoroughly before use.
2. Each Influenza B lysate sample, Influenza B nucleoprotein standard, blank, and control medium should be assayed in duplicate.
3. Add 100 µL of Influenza B lysate or Influenza B nucleoprotein standard to Anti-Influenza B Nucleoprotein Antibody Coated Plate.
4. Cover with a Plate Cover and incubate at 37°C for 2 hours.
5. Remove plate cover and empty wells. Wash microwell strips 5 times with 250 µL 1× 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 1× Wash Buffer.
6. Add 100 µL of the diluted FITC-Conjugated Anti-Influenza B 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.

Interpretation Of Results

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

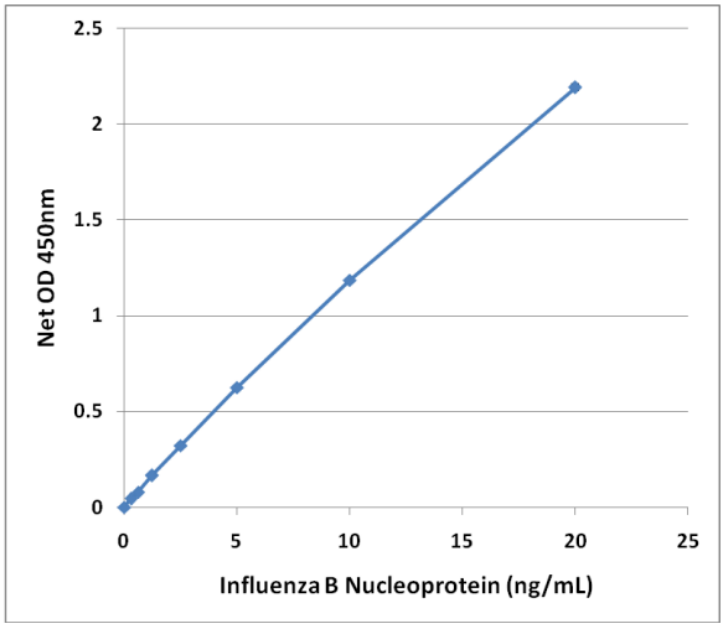

Figure 1: Influenza B Nucleoprotein ELISA Standard Curve.

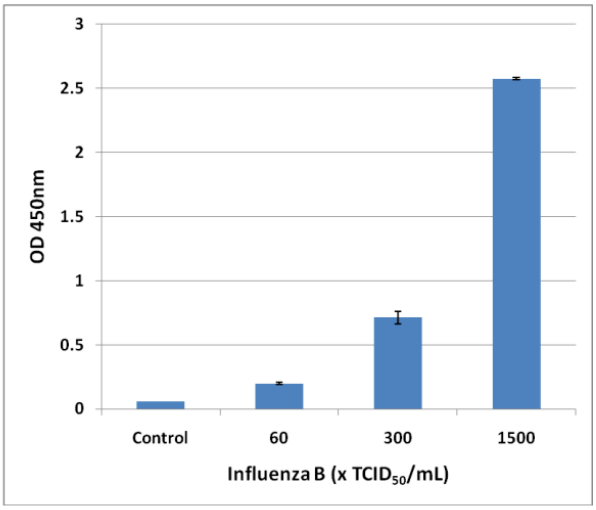

Figure 2: Influenza B Nucleoprotein in Influenza B Culture Fluid. Influenza B culture fluid (3.80 x 10^6 $TCID_{50}$/mL) was first diluted 100-fold with culture medium, then heat inactivated and lysed in Viral Lysis Buffer. Influenza B lysate was subjected to Influenza B Nucleoprotein ELISA Kit according to Assay Protocol.

Sensitivity

0.313 ng/mL

Specificity

The ELISA antibodies only recognize the nucleoprotein from Influenza B, will not react with the nucleoprotein from Influenza A nor Influenza C.

Precautions

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

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