

Avian Influenza A Nucleoprotein Antigen Capture ELISA Kit

Cat. No.:DEIA2026

Pkg.Size:2 x 96T

Intended use

This kit is a highly sensitive and specific enzyme immunoassay for the detection of Influenza A nucleoprotein antigen in complex sample matrices derived from both human and veterinary sources.

General Description

Influenza virus nucleoprotein (NP) is a structural protein which encapsidates the negative strand viral RNA. NP is one of the main determinants of species specificity. The question of how far the NP gene can cross the species barrier by reassortment and become adapted by mutation to the new host has been discussed.

Principle Of The Test

The qualitative immunoenzymatic determination of antibodies to Influenza A Nucleoprotein Antigen is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique. Microtiter strip wells are precoated with antigens to bind corresponding antibodies of the specimen. After washing the wells to remove all unbound sample material horseradish peroxidase (HRP) conjugate is added. This conjugate binds to the captured Influenza A Nucleoprotein Antigen antibodies. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product. The intensity of this product is proportional to the amount of antibodies in the specimen. Sulphuric acid is added to stop the reaction. Absorbance at 450 nm is read using an ELISA microwell plate reader.

Reagents And Materials Provided

1. Antigen Capture Plate (96 tests) 2 each
2. Sample Preparation Reagent (1x) 12 mL
3. Positive Control (1x) 1 mL
4. Negative Control (1x) 2 mL
5. Wash Buffer (20x) 30 mL
6. Detection Antibody, HRP-labeled (1x) 22 mL
7. Chromagen Solution (1x) 22 mL
8. Stop Solution (1x) 22 mL
9. Sample Dilution Tray 2 each

Materials Required But Not Supplied

1. Pipettes and pipette tips: 10 μ L - 20 μ L or 20 μ L - 100 μ L
2. Multi-channel Pipettes and pipette tips: 5 - 50 μ L and 50 - 300 μ L
3. Buffer and Reagent Reservoirs
4. Vortex Mixer
5. Deionized Water
6. Microtiter Plate Reader capable of reading absorbency at 450 nm and 590 nm
7. Orbital Microtiter Plate Shaker
8. Absorbent Paper or Cloth

Storage

Store all kit components at 2-8 °C. Crystal formation may occur in the wash buffer concentrate during prolonged storage at 2-8 °C

Assay Steps

1. Remove the kit components from storage and allow to warm to room temperature.
2. Determine the number of test wells needed. Use one well for each sample. In addition, include one well for the Positive Control and three wells for the Negative Control.
3. To begin the assay, transfer 50 µl of Sample Preparation Reagent to the appropriate number of wells in the dilution tray provided.
4. Add 200 µl of each sample, positive control, and negative control to the Sample Preparation Reagent. Mix by pipetting up and down several times.
5. Transfer 100 µl of sample or control to the appropriate wells of the Antigen Capture Plate.
6. Cover the plate and incubate for 30 minutes at room temperature on a plate shaker set at moderate speed.
7. Add 100 µl of 1x Detection Antibody to each well, cover the plate and incubate for 45 minutes on a plate shaker using the same settings (Step 6).
8. Wash the wells 6x with 1x Wash Buffer.
9. Add 100 µl of Chromagen Solution to each well and incubate for 10 minutes on a plate shaker.
10. Stop the reaction by the addition of 100 µl of Stop Solution.
11. Shake the plate for 10-15 sec. to ensure that the reaction is uniformly stopped and then read the plate in a plate reader using a 450 nm filter.

Sensitivity

<90%- <89%

Analyte Gene Information

Gene Name	NP nucleocapsid protein [Influenza A virus (A/Puerto Rico/8/1934(H1N1))]
Official Symbol	NP
Synonyms	NP; nucleocapsid protein;
GeneID	956531
Protein Refseq	NP_040982
Pathway	Assembly of Viral Components at the Budding Site, organism-specific biosystem; Budding, organism-specific biosystem; Disease, organism-specific biosystem; Entry of Influenza Virion into Host Cell via Endocytosis, organism-specific biosystem; Export of Viral Ribonucleoproteins from Nucleus, organism-specific biosystem; Fusion and Uncoating of the Influenza Virion, organism-specific biosystem; Fusion of the Influenza Virion to the Host Cell Endosome, organism-specific biosystem;