Influenza A Virus Nucleoprotein Antigen Capture ELISA

Product Insert

Photometric Format

Cat. No. IAV-142-2 (2 x 96 wells)

For Research Use Only

Introduction

Influenza viruses can be divided into three classes, A, B, and C, largely based upon conserved antigenic differences in the internal nucleoprotein. Influenza A virus, typically encountered more frequently than types B and C, and associated with the majority of serious epidemics, can be further subdivided into strains or subtypes based on antigenic differences in the external hemagglutinin proteins (H1-H16) and neuraminidase proteins (N1-N9).

A variety of wild waterfowl appear to be the predominant natural reservoir for Influenza A viruses and subtypes representing most of the hemagglutinin and neuraminidase combinations can be found circulating in these birds. Historically, human influenza virus infections have been associated with H1N1, N2N2, and H3N2 subtypes of influenza A, although a recent (1997) and significant outbreak in Hong Kong was identified as an H5N1 subtype. This outbreak was not only significant because it resulted in 18 human infections and 6 deaths, but it also represented the first known demonstration of avian influenza virus transmission to humans.

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While influenza A subtype identification is extremely important (vaccine production, epidemiology), the rapid and accurate differentiation of influenza A from influenza B and C and other respiratory agents in humans and animals is also important (treatment and biosecurity).

Virusys has developed 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. The assay can be completed in less than 1.5 hr. and contains only one wash step. In addition, the test kit incorporates proprietary diluents that are designed to prevent the development of nonspecific signal derived from complex sample matrix effects and/or the nonspecific adsorption of reactive test components which result in improvements in both sensitivity and specificity . The test kit is available in a standard photometric detection format (IAV142-2 or IAV142-5) or in a chemiluminescent detection format (IAV-143-2 or IAV-143-5) for enhanced sensitivity. The kit has been tested against a wide variety of influenza A subtypes for sensitivity and potentially interfering viruses and bacteria for specificity.

Components

1. IAV Antigen Capture Plate (96 tests)-2 ea.
2. Sample Preparation Reagent (1x)-12 ml
3. IAV Positive Control (1x)-1 ml
4. IAV Negative Control (1x)- 2 x 1.5 ml
5. Wash Buffer (20x)-30 ml
6. IAV Detection Antibody, HRP-labeled (1x)-22 ml
7. Chromagen Solution (1x)-22 ml
8. Stop Solution (1x)-22 ml
9. Sample Dilution Tray-2 ea.

Optional Components

IAV198 Influenza A NCP Antigen Calibration Kit

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. The crystals can be re-dissolved by swirling the bottle in warm tap water.

Procedure

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 IAV 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 **IAV Antigen Capture Plate**.
6. Cover the plate and incubate for 30 min. at room temperature on a plate shaker set at moderate speed.
7. Add 100 µl of **1x IAV Detection Antibody** to each well, cover the plate and incubate for 45 min. on a plate shaker using the same settings (Step 6).
8. Wash the wells 6x with at least 300 µl/well **1x Wash Buffer**.
9. Add 100 µl of **Chromagen** to each well and incubate for 10 min. 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.

Quality Control

1. All negative control absorbance values should be $\leq$0.250.
2. The positive control absorbance value should be $\geq$0.500.
3. The calculated value for the positive control/cut-off should be $\geq$2 (see below).

Determination of Cut-off and Interpretation of Results

1. To determine the **cut-off value**, calculate the mean of the three negative control absorbance values and multiply this value by 2.
2. To interpret the results for a given sample, divide the absorbance value for the sample by the cut-off value. Calculated sample values that are $>$1.1 are considered reactive. Calculated sample values that are $<$0.9 are considered nonreactive. Calculated sample values that are $\geq$0.9 and $\leq$1.1 are considered equivocal.

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