Version 12-07/24

User's Manual

Influenza A Nucleoprotein Antibody Inhibition ELISA Kit

REF **DEIAFY106**

Σ **2 x 96T**

RUO

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

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PRODUCT INFORMATION

Intended Use

A highly sensitive and specific enzyme-linked immunosorbent assay (ELISA) for the detection of influenza A NP-specific antibodies in serum from human and veterinary sources.

General Description

The assay utilizes"inhibition of binding" technology, it may be used with serum from any species, and therefore does not require speciesspecific conjugates. The assay can be completed in less than 1.5 hr., contains only one wash step, and incorporates proprietary diluents that are designed to prevent the development of nonspecific signal derived from sample matrix and/or the nonspecific adsorption of reactive test components. The result is an assay that is both highly sensitive and specific.

Reagents And Materials Provided

1. IAB Antigen Capture Plate (96 tests) - 2 ea.
2. IAB Antigen Diluent (1x) - 24 ml
3. IAB Antigen Concentrate (1x) - 4 ml
4. IAB Positive Serum Control (1x) - 0.1 ml
5. IAB Negative Serum Control (1x) - 0.1 ml
6. Wash Buffer (20x) - 1 x 50 ml
7. IAB Detection Antibody, HRP-labeled (1x) - 12 ml
8. Chromagen Solution (1x) - 22 ml
9. Stop Solution (1x) - 22 ml
10. Sample Dilution Tray - 5 ea.

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.

Assay 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 two wells each for the Antigen Diluent Control, Positive Serum Control and the Negative Serum Control.
3. Based upon the number of wells required for the assay, prepare Sample Diluent from the IAB Antigen Concentrate and IAB Antigen Diluent using a ratio of 1 ml of Antigen Concentrate to 5 ml Antigen Diluent. For example, if using the entire plate combine 2 ml of Antigen Concentrate with 10 ml of Antigen Diluent.
4. To begin the assay, transfer 5 µl of each serum control (two wells each) and each sample (one well each) to

the appropriate wells of the dilution tray.

5. Add 100 µl of IAB Sample Diluent to each sample, positive control, and negative control well. Mix by pipetting up and down several times.
6. Add 100 µl of the Antigen Diluent to the appropriate wells (NOTE: Wells containing Antigen Diluent DO NOT receive Sample Diluent).
7. Incubate for 10 min. at room temperature.
8. Transfer 50 µl of sample or control to the appropriate wells of the IAB Antigen Capture Plate.
9. Cover the plate and incubate for 30 min at room temperature on a plate shaker set at moderate speed.
10. Add 50 µl of 1x IAB Detection Antibody to each well. DO NOT WASH THE PLATE AT THIS TIME. Cover the plate and incubate for 30 min on a plate shaker using the same settings (Step 6).
11. Wash the wells 6x with 1x IAB Wash Buffer.
12. Add 100 µl of IAB Chromagen to each well and incubate for 10 min. on a plate shaker.
13. Stop the reaction by the addition of 100 µl of IAB Stop Solution.
14. 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. The negative serum control absorbance values should be ≥ 0.600.
2. The positive serum control absorbance values should be ≤ 0.300.
3. The diluent control absorbance values should be ≤ 0.300.
4. The mean absorbance value for the positive control serum should be ± 15% of the mean absorbance value for the diluent control.

Interpretation Of Results

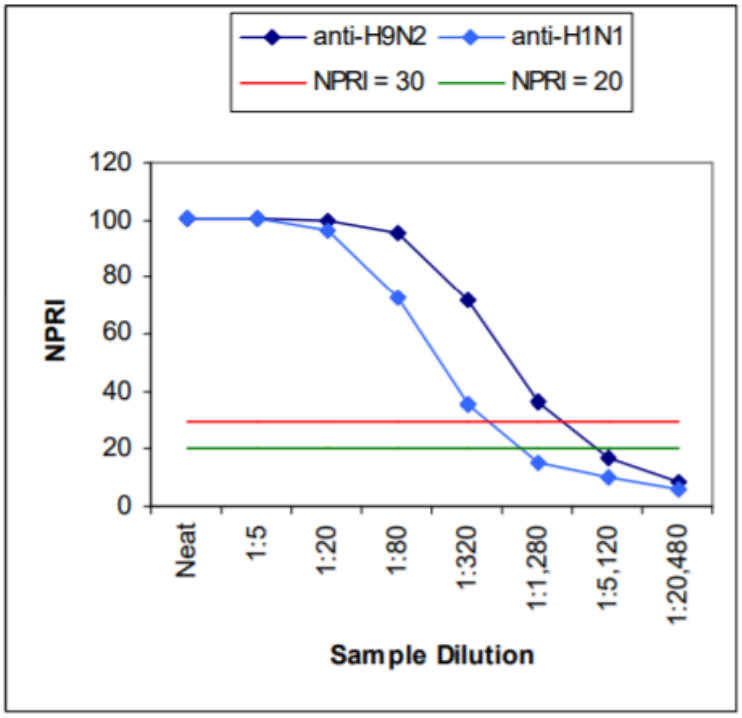
A positive result with the Influenza A Nucleoprotein Antibody Inhibition Test is dependent upon the presence of NP-specific antibody in the sample. Binding of the antibody in the sample to NP antigen in the sample diluent will result in the complete or partial inhibition of NP binding to the antibody coated on the IAB Antigen Capture Plate. The resulting decrease in NP binding can then be used to calculate a NCP Reduction Index for each sample.

1. To calculate the NPRI for each sample, it is first necessary to calculate the mean absorbance values for the diluent control and the positive and negative controls.
2. Subtract the mean value of the diluent control from the mean of the positive serum control (maximal NP removal) and from the mean of the negative serum control (minimal NP removal) and the absorbance values for all samples.

Sensitivity

Titration of anti-H9N2 and anti-H1N1 positive chicken sera. (NOTE: sample dilutions represent dilution of positive sera in pool of normal [negative] chicken serum.

Each dilution was then diluted 1:21 into IAB Sample Diluent for assay as described above).



Specificity

NPRI values for hyperimmune sera obtained from SPF chickens immunized with the following viruses (all NPRI<10):

Infectious Bronchitis Virus (Ark): 0

Infectious Bursal Disease Virus: 7

Infectious Laryngotracheitis Virus: 7

Newcastle Disease Virus: 4

Marek's Disease Virus: 4

Avian Adenovirus (Type 1): 1