



# Influenza A IgG ELISA

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**NB-06-0274**



## ***Influenza A IgG ELISA***

*Cat# NB-06-0274*

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## 1. Intended Use

The Neo Biotech Influenza A IgG Antibody ELISA Test Kit has been designed for the the detection and the quantitative determination of specific IgG antibodies against Influenza A in serum and plasma. Further applications in other body fluids are possible and can be requested from the Technical Service of Neo Biotech.

This assay is intended for research use only.

## 2. Principle of the Test

The Neo Biotech Influenza A IgG antibody test kit is based on the principle of the enzyme immunoassay (EIA). Influenza A antigen is bound on the surface of the microtiter strips. Diluted sample serum or ready-to-use standards are pipetted into the wells of the microtiter plate. A binding between the IgG antibodies of the serum and the immobilized Influenza A antigen takes place. After a one hour incubation at room temperature, the plate is rinsed with diluted wash solution, in order to remove unbound material. Then ready-to-use anti-human-IgG peroxidase conjugate is added and incubated for 30 minutes. After a further washing step, the substrate (TMB) solution is pipetted and incubated for 20 minutes, inducing the development of a blue dye in the wells. The color development is terminated by the addition of a stop solution, which changes the color from blue to yellow. The resulting dye is measured spectrophotometrically at the wavelength of 450 nm. The concentration of the IgG antibodies is directly proportional to the intensity of the color.

## 3. Limitations, Precautions and General Comments

- ☒ Only for research use! Do not ingest or swallow! The usual laboratory safety precautions as well as the prohibition of eating, drinking and smoking in the lab have to be followed.
- ☒ All sera and plasma or buffers based upon, have been tested respective to HBsAg, HIV and HCV with recognized methods and were found negative. Nevertheless precautions like the use of latex gloves have to be taken.
- ☒ Serum and reagent spills have to be wiped off with a disinfecting solution (e.g. sodium hypochlorite, 5%) and have to be disposed of properly.
- ☒ All reagents have to be brought to room temperature (18 to 25 °C) before performing the test.
- ☒ Before pipetting all reagents should be mixed thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided.
- ☒ It is important to pipet with constant intervals, so that all the wells of the microtiter plate have the same conditions.
- ☒ When removing reagents out of the bottles, care has to be taken that the stoppers are not contaminated. Further a possible mix-up has to be avoided. The content of the bottles is usually sensitive to oxidation, so that they should be opened only for a short time.

- ☒ In order to avoid a carry-over or a cross-contamination, separate disposable pipet tips have to be used.
- ☒ No reagents from different kit lots have to be used, they should not be mixed among one another.
- ☒ All reagents have to be used within the expiry period.
- ☒ In accordance with a Good Laboratory Practice (GLP) or following ISO9001 all laboratory devices employed should be regularly checked regarding the accuracy and precision. This refers amongst others to microliter pipets and washing or reading (ELISA-Reader) instrumentation.
- ☒ The contact of certain reagents, above all the stopping solution and the substrate with skin, eye and mucosa has to be avoided, because possible irritations and acid burns could arise, and there exists a danger of intoxication.

#### 4. Reagents Provided

Store kit components at 2-8°C and do not use after the expiry date on the box outer label. Before use, all components should be allowed to warm up to ambient temperature (18-25°C). After use, the plate should be resealed, the bottle caps replaced and tightened and the kit stored at 2-8°C. The opened kit should be used within three months.

Components	Volume / Qty.
<b>Influenza A antigen coated microtiter strips</b>	<b>12</b>
<b>Calibrator A (Negative Control)</b>	<b>2 mL</b>
<b>Calibrator B (Cut-Off Standard)</b>	<b>2 mL</b>
<b>Calibrator C (Weak Positive Control)</b>	<b>2 mL</b>
<b>Calibrator D (Positive Control)</b>	<b>2 mL</b>
<b>Enzyme Conjugate</b>	<b>15 mL</b>
<b>Substrate</b>	<b>15 mL</b>
<b>Stop Solution</b>	<b>15 mL</b>
<b>Sample Diluent</b>	<b>60 mL</b>
<b>Washing Buffer (10X)</b>	<b>60 mL</b>
<b>Plastic foils</b>	<b>2</b>
<b>Plastic bag</b>	<b>1</b>

#### **4.1. Microtiter Strips**

12 strips with 8 breakable wells each, coated with a Influenza A antigen (strains Beijing 265/95 (H1N1) and Sydney 5/97 (H3N2)). Ready-to-use.

#### **4.2. Calibrator A (Negative Control)**

2 mL, protein solution diluted with PBS, contains no IgG antibodies against Influenza A. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

#### **4.3. Calibrator B (Cut-Off Standard)**

2 mL human serum diluted with PBS, contains a low concentration of IgG antibodies against Influenza A. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

#### **4.4. Calibrator C (Weak Positive Control)**

2 mL, human serum diluted with PBS, contains a medium concentration of IgG antibodies against Influenza A. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

#### **4.5. Calibrator D (Positive Control)**

2 mL, human serum diluted with PBS, contains a high concentration of IgG antibodies against Influenza A. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

#### **4.6. Enzyme Conjugate**

15 mL, anti-human-IgG-HRP (rabbit), in protein-containing buffer solution. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane and 5 mg/L Proclin™. Ready-to-use.

#### **4.7. Substrate**

15 mL, TMB (tetramethylbenzidine). Ready-to-use.

#### **4.8. Stop Solution**

15 mL, 0.5 M sulfuric acid. Ready-to-use.

#### **4.9. Sample Diluent**

60 mL, PBS/BSA buffer. Addition of 0.095 % sodium azide. Ready-to-use.

#### **4.10. Washing Buffer**

60 mL, PBS + Tween 20, 10x concentrate. Final concentration: dilute 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.

#### **4.11. Plastic Foils**

2 pieces to cover the microtiter strips during the incubation.

#### **4.12. Plastic Bag**

Resealable, for the dry storage of non-used strips.

### **5. Materials Required but not Provided**

- ☐ 5 µL-, 100 µL- and 500 µL micro- and multichannel pipets
- ☐ Microtiter Plate Reader (450 nm)
- ☐ Microtiter Plate Washer

- ☒ Reagent tubes for the serum dilution
- ☒ Bidistilled water

## 6. Specimen Collection and Handling

Principally serum or plasma (EDTA, heparin) can be used for the determination. Serum is separated from the blood, which is aseptically drawn by venipuncture, after clotting and centrifugation. The serum or plasma samples can be stored refrigerated (2-8°C) for up to 48 hours, for a longer storage they should be kept at -20 °C. The samples should not be frozen and thawed repeatedly. Lipemic, hemolytic or bacterially contaminated samples can cause false positive or false negative results.

For the performance of the test the samples (not the standards) have to be diluted 1:101 with ready-to-use sample diluent (e.g. 5 µL serum + 500 µL sample diluent).

## 7. Assay Procedure

### 7.1. Preparation of Reagents

**Washing Solution:** dilute before use 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.

- ☒ Strict adherence to the protocol is advised for reliable performance. Any changes or modifications are the responsibility of the user.
- ☒ All reagents and samples must be brought to room temperature before use, but should not be left at this temperature longer than necessary.
- ☒ Standards and samples should be assayed in duplicates.
- ☒ A standard curve should be established with each assay.
- ☒ Return the unused microtiter strips to the plastic bag and store them dry at 2-8°C.

### 7.2. Assay Steps

1. Prepare a sufficient amount of microtiter wells for the standards, controls and samples in duplicate as well as for a substrate blank.
2. Pipet 100 µL each of the **diluted** (1:101) samples and the **ready-to-use** standards and controls respectively into the wells. Leave one well empty for the substrate blank.
3. Cover plate with the enclosed foil and incubate at room temperature for 60 minutes.
4. Empty the wells of the plate (dump or aspirate) and add 300 µL of diluted washing solution. This procedure is repeated totally three times. Rests of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.

5. Pipet 100  $\mu$ L each of ready-to-use conjugate into the wells. Leave one well empty for the substrate blank.
6. Cover plate with the enclosed foil and incubate at room temperature for 30 minutes.
7. Empty the wells of the plate (dump or aspirate) and add 300  $\mu$ L of diluted washing solution. This procedure is repeated totally three times. Rests of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.
8. Pipet 100  $\mu$ L each of the ready-to-use substrate into the wells. This time also the substrate blank is pipetted.
9. Cover plate with the enclosed foil and incubate at room temperature for 20 minutes in the dark (e.g. drawer).
10. To terminate the substrate reaction, pipet 100  $\mu$ L each of the ready-to-use stop solution into the wells. Pipet also the substrate blank.
11. After thorough mixing and wiping the bottom of the plate, perform the reading of the absorption at 450 nm (optionally reference wavelength of 620 nm). The color is stable for at least 60 minutes.

## 8. Evaluation

The mean values for the measured absorptions are calculated after subtraction of the substrate blank value. The difference between the single values should not exceed 10%.

### Example

	OD Value	corrected OD	Mean OD Value
Substrate Blank	0.011		
Negative Control	0.027 / 0.023	0.016 / 0.012	0.014
Cut-Off Standard	0.650 / 0.637	0.639 / 0.626	0.633
Weak Positive Control	1.431 / 1.442	1.420 / 1.431	1.426
Positive Control	2.241 / 2.279	2.230 / 2.268	2.249

The above table contains only an example, which was achieved under arbitrary temperature and environmental conditions. The described data constitute consequently **no reference values** which have to be found in other laboratories in the same way.

### 8.1. Quantitative Evaluation

The ready-to-use standards and controls of the Influenza A antibody kit are defined and expressed in arbitrary units (U/mL). This results in an exact and reproducible quantitative evaluation. The values for controls and standards in units are printed on the labels of the vials.

For a quantitative evaluation the absorptions of the standards and controls are graphically drawn against their concentrations. From the resulting reference curve the concentration values for each sample can then be extracted in relation to their absorptions. It is also possible to use automatic computer programs.

## 10. Assay Characteristics

Influenza A ELISA	IgG	IgA	IgM
Intra-Assay-Precision	8.5 %	8.8 %	8.3 %
Inter-Assay-Precision	6.5 %	13.6 %	10.8 %
Inter-Lot-Precision	3.8 – 6.1 %	4.6 – 15.5 %	1.3 – 9.9 %
Recovery	100 – 114 %	93 – 124 %	83 – 90 %
Linearity	79 – 114 %	75 – 128 %	73 – 122 %
Cross-Reactivity	No cross-reactivity to RSV, Adenovirus and Parainfluenza 1/2/3.		
Interferences	No interferences to bilirubin up to 0.3 mg/mL, hemoglobin up to 8.0 mg/mL and triglycerides up to 5.0 mg/mL.		

## 11. References

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