Influenza A IgG ELISA

For the quantitative determination of IgG-class antibodies against Influenza A virus in human serum or plasma (citrate).

For Research use Only. Not For Use In Diagnostic Procedures.

Catalog No.: 20-IFAHU-E01
Size: 96 Wells
Version: (5.1) 01 Dec 15-ALPCO December 4, 2015
INTENDED USE
The Influenza Virus A IgG-ELISA is for quantitative determination of IgG class antibodies against Influenza virus A in human serum or plasma (citrate). This product is for research use only.

PRINCIPLE OF THE ASSAY
The immunoenzymatic determination of IgG-class antibodies against Influenza Virus A is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.

Microtiter strip wells are precoated with Influenza Virus A antigens to bind corresponding antibodies of the specimen. After washing the wells to remove all unbound sample material horseradish peroxidase (HRP) labelled anti-human IgG conjugate is added. This conjugate binds to the captured Influenza Virus A-specific 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 Influenza Virus A specific IgG antibodies in the sample. Sulfuric acid is added to stop the reaction. This produces a yellow endpoint color. Absorbance at 450 nm is read using an ELISA microwell plate reader.

MATERIALS

Reagents supplied
Influenza Virus A Coated Wells (IgG): 12 breakaway 8-well snap-off strips coated with Influenza Virus A antigen; in resealable aluminum foil.
IgG Sample Diluent ***: 1 bottle containing 100 ml of buffer for sample dilution; pH 7.2 ± 0.2; colored yellow; ready to use; white cap.
Stop Solution: 1 bottle containing 15 ml sulfuric acid, 0.2 mol/l; ready to use; red cap.
Washing Solution (20x conc.)*: 1 bottle containing 50 ml of a 20-fold concentrated buffer (pH 7.2 ± 0.2) for washing the wells; white cap.
Influenza Virus A anti-IgG Conjugate**: 1 bottle containing 20 ml of peroxidase labelled rabbit antibody to human IgG.; colored blue, ready to use; black cap.
TMB Substrate Solution: 1 bottle containing 15 ml 3,3',5,5'-tetramethylbenzidine (TMB); ready to use; yellow cap.
Influenza Virus A IgG High Control***: 1 bottle containing 2 ml; colored yellow; ready to use; red cap.
Influenza Virus A IgG Calibrator***: 1 bottle containing 3 ml; colored yellow; ready to use; green cap.
Influenza Virus A IgG Low Control***: 1 bottle containing 2 ml; colored yellow; ready to use; blue cap.
* contains 0.1 % Bronidox L after dilution
** contains 0.2 % Bronidox L
*** contains 0.1 % Kathon

Materials Supplied
− 1 Strip holder
Materials and Equipment needed

- ELISA microwell plate reader, equipped for the measurement of absorbance at 450/620nm
- Incubator 37°C
- Manual or automatic equipment for rinsing wells
- Pipettes to deliver volumes between 10 and 1000 µl
- Vortex tube mixer
- Deionized or (freshly) distilled water
- Disposable tubes
- Timer

STABILITY AND STORAGE
The reagents are stable up to the expiry date stated on the label when stored at 2-8°C.

REAGENT PREPARATION

It is very important to bring all reagents, samples and controls to room temperature (20-25°C) before starting the test run!

Coated snap-off Strips
The ready to use breakaway snap-off strips are coated with Influenza Virus A antigen. Store at 2-8°C.
Immediately after removal of strips, the remaining strips should be resealed in the aluminum foil along with the desiccant supplied and stored at 2-8°C; stability until expiry date.

Influenza Virus A anti-IgG Conjugate
The bottle contains 20 ml of a solution with anti-human-IgG horseradish peroxidase, buffer, stabilizers, preservatives and an inert blue dye. The solution is ready to use.
Store at 2-8°C. After first opening stability until expiry date when stored at 2-8°C.

Controls
The bottles labelled with High Control, Calibrator and Low Control contain a ready to use control solution. It contains 0.1% Kathon and has to be stored at 2-8°C.
After first opening stability until expiry date when stored at 2-8°C.

IgG Sample Diluent
The bottle contains 100 ml phosphate buffer, stabilizers, preservatives and an inert yellow dye. It is used for the dilution of the sample. This ready to use solution has to be stored at 2-8°C.
After first opening stability until expiry date when stored at 2-8°C.

Washing Solution (20x conc.)
The bottle contains 50 ml of a concentrated buffer, detergents and preservatives.
Dilute washing solution 1+19; e.g. 10 ml washing solution + 190 ml fresh and germ free redistilled water. The diluted buffer will keep for 5 days if stored at room temperature. Crystals in the solution disappear by warming
up to 37 °C in a water bath.

After first opening the concentrate is stable until the expiry date.

**TMB Substrate Solution**
The bottle contains 15 ml of a tetramethylbenzidine/hydrogen peroxide system. The reagent is ready to use and has to be stored at 2-8°C, away from the light. The solution should be colorless or have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be discarded.

*After first opening stability until expiry date when stored at 2-8°C.*

**Stop Solution**
The bottle contains 15 ml 0.2 M sulfuric acid solution (R 36/38, S 26). This ready to use solution has to be stored at 2-8°C. *After first opening stability until expiry date.*

**SAMPLE COLLECTION AND PREPARATION**
Use human serum or plasma (citrate) samples with this assay. If the assay is performed within 5 days after sample collection, the sample should be kept at 2-8°C; otherwise they should be aliquotted and stored deep-frozen (-20 to -70°C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing. Heat inactivation of samples is not recommended.

**Sample Dilution**
Before assaying, all samples should be diluted 1+100 with IgG Sample Diluent. Dispense 10µl sample and 1ml IgG Sample Diluent into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

**ASSAY PROCEDURE**

**Test Preparation**
Please read the test protocol carefully before performing the assay. Result reliability depends on strict adherence to the test protocol as described.

The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems it is recommended to increase the washing steps from three to five and the volume of washing solution from 300µl to 350µl to avoid washing effects.

Prior to commencing the assay, the distribution and identification plan for all samples and controls should be carefully established on the result sheet supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Please allocate at least:
1 well (e.g. A1) for the substrate blank,
1 well (e.g. B1) for the low control,
2 wells (e.g. C1+D1) for the calibrator and
1 well (e.g. E1) for the high control.

*It is recommended to determine controls and samples in duplicate if necessary.*

Perform all assay steps in the order given and without any appreciable delays between the steps.

A clean, disposable tip should be used for dispensing each control and sample.

Adjust the incubator to 37° ± 1°C.
1. Dispense 100µl controls and diluted samples into their respective wells. Leave well A1 for substrate blank.
2. Cover wells with the foil supplied in the kit.
3. Incubate for 1 hour ± 5 min at 37±1°C.
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300µl of Washing Solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be >5sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!
   **Note:** Washing is critical! Insufficient washing results in poor precision and falsely elevated absorbance values.
5. Dispense 100µl Influenza Virus A anti IgG Conjugate into all wells except for the blank well (e.g. A1). Cover with foil
6. Incubate for 30 min at room temperature. Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100µl TMB Substrate Solution into all wells
9. Incubate for exactly 15 min at room temperature in the dark.
10. Dispense 100µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution. Any blue color developed during the incubation turns into yellow.
   **Note:** High samples can cause dark precipitates of the chromogen! These precipitates have an influence when reading the optical density. Pre-dilution of the sample with physiological sodium chloride solution, for example 1+1, is recommended, and then dilute 1+100 with dilution buffer and multiply the results in DU by 2.
11. Measure the absorbance of the sample at 450/620 nm within 30 min after addition of the Stop Solution.

**Measurement**
Adjust the ELISA Microwell Plate Reader to zero using the substrate blank in well A1.
If - due to technical reasons - the ELISA reader cannot be adjusted to zero using the substrate blank in well A1, subtract the absorbance value of well A1 from all other absorbance values measured in order to obtain reliable results.
Measure the absorbance of all wells at **450 nm** and record the absorbance values for each control and sample in the distribution and identification plan.
Dual wavelength reading using 620 nm as reference wavelength is recommended.
Where applicable calculate the **mean absorbance values** of all duplicates.

**RESULTS**

**Run Validation Criteria**
In order for an assay to be considered valid, the following criteria must be met:
- **Substrate blank** in A1: Absorbance value < **0.100**.
- **Low control** in B1: Absorbance value < **0.200** and < **calibrator**
- **Calibrator** in C1 and D1: Absorbance value **0.150 – 1.30**.
- **High control** in E1: Absorbance value > **calibrator**
If these criteria are not met, the test is not valid and must be repeated.

**Calculation of Results**
The calibrator is the mean absorbance value of the calibrator determinations.

**Interferences**
Interferences with hemolytic, lipemic, or icteric sera are not observed up to a concentration of 10mg/ml hemoglobin, 5 mg/ml triglycerides, and 0.2 mg/ml bilirubin

**Limitations of the Procedure**
Bacterial contamination or repeated freeze-thaw cycles of the samples may affect the absorbance values.

**Precautions and Warnings**
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious.
- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette samples and dispense conjugate without splashing accurately to the bottom of wells.
- The ELISA is only designed for qualified personnel who are familiar with good laboratory practice.

**WARNING:** In the used concentration Bronidox L has hardly any toxicological risk upon contact with skin and mucous membranes!

**WARNING:** Sulfuric acid irritates eyes and skin. Keep out of the reach of children. Upon contact with the eyes, rinse thoroughly with water and consult a doctor!

**Disposal Considerations**
Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact the local authorities or waste management companies which will give advice on how to dispose hazardous waste.
**BIBLIOGRAPHY**


**SCHEME OF THE ASSAY**

**Test Preparation**

Prepare reagents and samples as described. Establish the distribution and identification plan for all samples and controls on the result sheet supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

**Assay Procedure**

<table>
<thead>
<tr>
<th>Substrate blank (e.g. A1)</th>
<th>Low control</th>
<th>High control</th>
<th>Calibrator</th>
<th>Sample (diluted 1+100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low control</td>
<td>100µl</td>
<td></td>
<td>-</td>
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<tr>
<td>High control</td>
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<tr>
<td>Sample (diluted 1+100)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100µl</td>
</tr>
</tbody>
</table>

Cover wells with foil supplied in the kit

**Incubate for 1 hour at 37°C**

Wash each well three times with 300µl of washing solution

<table>
<thead>
<tr>
<th>Conjugate</th>
<th>100µl</th>
<th>100µl</th>
<th>100µl</th>
<th>100µl</th>
<th>100µl</th>
</tr>
</thead>
</table>

Cover wells with foil supplied in the kit

**Incubate for 30 min at room temperature**

Wash each well three times with 300µl of washing solution

<table>
<thead>
<tr>
<th>TMB Substrate</th>
<th>100µl</th>
<th>100µl</th>
<th>100µl</th>
<th>100µl</th>
<th>100µl</th>
</tr>
</thead>
</table>

**Incubate for exactly 15 min at room temperature in the dark**

<table>
<thead>
<tr>
<th>Stop Solution</th>
<th>100µl</th>
<th>100µl</th>
<th>100µl</th>
<th>100µl</th>
<th>100µl</th>
</tr>
</thead>
</table>

Photometric measurement at 450 nm (reference wavelength: 620 nm)