Diphtheria Toxoid IgG ELISA Kit

Prod. No.: DEIA328
Pkg. Size: 96T

INTENDED USE
The Diphtheria Toxoid IgG Antibody ELISA Test Kit has been designed for the detection and the quantitative determination of specific IgG antibodies against Diphtheria Toxoid in serum and plasma. Further applications in other body fluids are possible and can be requested from the Technical Service. This assay is intended for in-vitro diagnostic use only. Laboratory results can never be the only base of a medical report. The patient history and further tests have additionally to be taken into account.

GENERAL DESCRIPTION
Diphtheria is a bacterial infectious disease which appears predominantly during the childhood. The disease leads particularly to an inflammation of the pharynx, larynx and nasal mucosa. Additionally, bacterial toxins cause via long-distance effect damages of the heart, circulation and CNS. Only the toxigenic strains are pathogenic. The etiologic agent is the Corynebacterium diphtheriae. These gram-positive bacteria prefer a microaerophil to anaerobe environment. Its pathogenicity is based on the secretion of an exotoxin that is circulat-ting in the blood and effecting the heart muscle, kidneys and CNS. The Diphtheria toxoid will be produced by lysogenic strains. Depending on the stage of disease, the three types ‘slight, middle and serious’ can be distinguished. The natural source of infection is the sick individual, whereas a carrier not absolutely shows symptoms. The infection is spread both through the aerial-droplet route and rarely by milk or smear infection. The appearance of Diphtheria shows a seasonal prevalence with the greatest incidence in winter. Especially non-vaccinated children will be infected. The incubation time is depending on the number of invasive germs. The place of infection is the mucosa of the respiratory tract, where an acute local infection is developing. The secreted toxin leads to a superficial inflammation of the mucosa associated with the formation of a brown film (pseudo-membrane) upon it, consisting of bacteria, necrotic epithelial cells, fibrin, red and white cells. From this local inflammation, the toxin reaches other organs by using the blood and lymphatic circulation. Here it may cause severe damages. The grade of disease depends on the immunostate of the child. Usually, a limited Diphtheria arises, whereas in case of an immunosuppression, a severe Diphtheria is observed. As a result of this disease course, patients may die. In most cases children will be vaccinated (e.g. DTP = Diphtheria-Tetanus-Pertussis) after the third month of life. The state of immunity can be monitored by determining the antitoxin IgG.

PRINCIPLE OF THE TEST
The Diphtheria Toxoid IgG antibody test kit is based on the principle of the enzyme immunoassay (EIA). Diphtheria Toxoid antigen is bound on the surface of the microtiter strips. Diluted patient 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 Diphtheria Toxoid 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.

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.

Microtiter Strips: 12 strips with 8 breakable wells each, coated with a Diphtheria Toxoid antigen. Ready-to-use.

Standards: 5 x 2 mL, human serum diluted with PBS, with 0, 0.01, 0.1, 0.5 and 1 IU/mL of IgG antibodies against Diphtheria Toxoid. Addition of 0.01 % methylisothiazolone and 0.01 % brononitrodioxane. Ready-to-use.

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

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

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

Sample Diluent: 60 mL, PBS/BSA buffer. Addition of 0.1 % sodium azide. Ready-to-use.

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.

Plastic Foils: 2 pieces to cover the microtiter strips during the incubation.

Plastic Bag: Resealable, for the dry storage of non-used strips.
SPECIMEN COLLECTION AND HANDLING

1. 5 μL-, 100 μL- and 500 μL micro- and multichannel pipets
2. Microtiter Plate Reader (450 nm)
3. Microtiter Plate Washer
4. Reagent tubes for the serum dilution
5. Bidistilled water

MATERIALS REQUIRED BUT NOT PROVIDED

Principally serum or plasma (EDTA, citrate) 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 (4-8°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 calibrators) have to be diluted 1:101 with ready-to-use sample diluent (e.g. 5 μL serum + 500 μL sample diluent).

ASSAY PROCEDURE

1. Reagent And Sample Preparation

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.
1) Strict adherence to the protocol is advised for reliable performance. Any changes or modifications are the responsibility of the user.
2) All reagents and samples must be brought to room temperature before use, but should not be left at this temperature longer than necessary.
3) Standard and samples should be assayed in duplicates.
4) A standard curve should be established with each assay.
5) Return the unused microtiter strips to the plastic bag and store them dry at 4-8°C.

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 substrate 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 30 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 μL 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 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.
8) Pipet 100 μL 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 μL 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.

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%.

1. Quantitative Evaluation

The ready-to-use standards of the Diphtheria Toxoid antibody kit are defined and expressed in International Units (IU/mL). This results in an exact and reproducible quantitative evaluation. Consequently for a given patient follow-up controls become possible. The values for the standards in International Units are printed on the labels of the vials. For a quantitative evaluation the absorptions of the standards are graphically drawn against their concentrations. From the resulting reference curve the concentration values for each patient sample can then be extracted in relation to their absorptions. It is also possible to use automatic computer programs. The dilution factor of the samples (1:101) has already been reconsidered in the concentration given for the standards.

2. Interpretation

The results of each patient sample can be assessed as follows:

- < 0.01 IU/mL  basic immunisation recommended
- 0.01 – 0.1 IU/mL booster vaccination recommended
- > 0.1 IU/mL  good immunity
ASSAY CHARACTERISTICS

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
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<tbody>
<tr>
<td>Diphtheria Toxoid ELISA</td>
<td>IgG</td>
</tr>
<tr>
<td>Intra-Assay-Precision</td>
<td>7.5 %</td>
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<tr>
<td>Inter-Assay-Precision</td>
<td>4.9 %</td>
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<tr>
<td>Inter-Lot-Precision</td>
<td>2.3 – 7.4 %</td>
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<tr>
<td>Analytical Sensitivity</td>
<td>0.004 U/mL</td>
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<tr>
<td>Recovery</td>
<td>96 – 102 %</td>
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<tr>
<td>Linearity</td>
<td>78 – 133 %</td>
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<tr>
<td>Clinical Specificity</td>
<td>94 %</td>
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<tr>
<td>Clinical Sensitivity</td>
<td>94 %</td>
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<tr>
<td>Cross-Reactivity: No cross-reactivity to Clostridium tetani</td>
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<tr>
<td>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</td>
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REFERENCES