Human Diphtheria IgG antibody ELISA Kit

Enzyme Immunoassay for the quantification of Diphtheria Toxoid IgG in serum and plasma

Catalog number: ARG80540

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INTRODUCTION

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 circulating 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 ASSAY

This assay employs the quantitative enzyme immunoassay technique. A specific Diphtheria Toxoid antigen has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Diphtheria Toxoid antibody present is bound by the immobilized antigen. After washing away any unbound substances, an HRP-conjugated antibody specific for human IgG is added to each well and incubate. Following the washing of any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of antigen-antibody binding in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm.The concentration of Diphtheria Toxoid IgG in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antigen-coated microplate	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
Standards	5 vials (0,0.01,0.1,0.5,1 IU/ml) (2ml each), (Ready-to-use)	4°C
HRP-conjugated antibody	15ml (Ready-to-use)	4°C
Sample Diluent	60ml	4°C
10X Wash buffer	60ml	4°C
TMB substrate	15ml	4°C (Protect from light)
STOP solution	15ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.

- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- If crystals are observed in the 10X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

For the performance of the test, the samples have to be diluted 1:101 with sample diluent.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X Wash buffer into distilled water to yield 1X Wash buffer.
- Patient sample: Dilute patient sample 1:101 with Sample diluent buffer before assay, mix well. (e.g. 5 µl of serum + 500 µl of sample diluent buffer)

  Note: the controls / standards are ready-to-use and need not further dilution.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100 µl of controls, diluted samples (1:101) and blank controls (sample diluent buffer) into wells. Incubate for 1h at RT.
3. Aspirate each well and wash, repeating the process 4 times for a total 5 washes. Wash by filling each well with 1× Wash Buffer (300 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add 100 µl HRP-conjugated antibody (ready-to-use) into each well (Except for blank well). Cover wells and incubate for 30 minutes at RT.
5. Aspirate each well and wash as step 3.

6. Add 100 µl of TMB Reagent to each well (Except for blank well). Incubate for 20 minutes at room temperature.
7. Add 100 µl of Stop Solution to each well (Except for blank well). The color of the solution should change from blue to yellow.
8. Read the OD with a microplate reader at 450nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

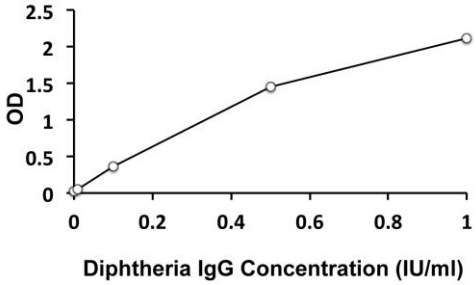
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The mean MDD was 0.004 U/ml.

Assay Range: 0.01-1.01 U/ml

Specificity

No cross reactivity was observed with the following factors:

Clostridium tetani

Intra-assay and Inter-assay precision

The CV value of intra-assay precision is 7.5 % and inter-assay precision is 4.9%.

Recovery

96-102%

Inter-lot Precision

2.3-7.4%

Linearity

78-133%