



User's Manual

Diphtheria IgG ELISA

Enzyme immunoassay for the detection and determination of human IgG antibodies against Diphtheria Toxoid in serum and plasma



For in-vitro diagnostic use only



IB79219



96 wells

CONTENTS

1. INTENDED USE	3
2. GENERAL INFORMATION	3
3. PRINCIPLE OF THE TEST	3
4. LIMITATIONS, PRECAUTIONS AND GENERAL COMMENTS	4
5. REAGENTS PROVIDED	4
6. MATERIALS REQUIRED BUT NOT PROVIDED	5
7. SAMPLE COLLECTION AND HANDLING	5
8. ASSAY PROCEDURE	6
9. EVALUATION	7
10. ASSAY CHARACTERISTICS	7
11. REFERENCES	8
SYMBOLS USED WITH IBL-AMERICA ASSAYS	9

1. 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. For *in-vitro* diagnostic use. 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

2. GENERAL INFORMATION

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-drop-let 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.

3. 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.

4. LIMITATIONS, PRECAUTIONS AND GENERAL COMMENTS

- Only for in-vitro 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.
- 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.

5. REAGENTS PROVIDED

Symbol	Components	Volume / Qty.
SORB MT	Diphtheria Toxoid antigen coated microtiter strips	12
CAL A	Calibrator A	2 mL
CAL B	Calibrator B	2 mL
CAL C	Calibrator C	2 mL
CAL D	Calibrator D	2 mL
CAL E	Calibrator E	2 mL
ENZ CONJ	Enzyme Conjugate	15 mL
SUB TMB	Substrate	15 mL
STOP SOLN	Stop Solution	15 mL
SAM DIL	Sample Diluent	60 mL
WASH SOLN 10x	Washing Buffer (10x)	60 mL

Storage and Stability (refer to the expiry date on the outer box label)

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. After the first opening the kit should be used within 3 months, the diluted wash buffer can be kept for 4 weeks at 2-8°C.

5.1. Microtiter Strips

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

5.2. 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 % bromonitrodioxane. Ready-to-use.

5.3. 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.

5.4. Substrate

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

5.5. Stop Solution

15 mL, 1 N acidic solution. Ready-to-use.

5.6. Sample Diluent

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

5.7. Washing Buffer

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

6. 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
- Deionized water
- Resealable Plastic Bag for the dry storage of non-used strips
- Re-usable black lid for covering

7. SAMPLE 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 7 days. 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).

8. ASSAY PROCEDURE

8.1. Preparation of Reagents

Washing Solution: dilute before use 1+9 with deionized 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.
- 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.

8.2. Assay Steps

1. Prepare a sufficient amount of microtiter wells for the standards, controls and samples 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 re-usable plate cover 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 µL each of ready-to-use conjugate into the wells. Leave one well empty for the substrate blank.
6. Cover plate with the re-usable plate cover 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 re-usable plate cover 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.

9. EVALUATION

Example

	OD Value	Corrected OD
Substrate Blank	0.016	
Standard 1 (0 IU/mL)	0.037	0.021
Standard 2 (0.01 IU/mL)	0.072	0.056
Standard 3 (0.1 IU/mL)	0.376	0.360
Standard 4 (0.5 IU/mL)	1.480	1.464
Standard 5 (1 IU/mL)	2.149	2.133

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.

9.1. Quantitative Evaluation

The ready-to-use standards of the Diphtheria Toxoid antibody kit are defined and expressed in International Units (IU/mL) and are calibrated against the **WHO reference preparation 04/496**. This results in an exact and reproducible quantitative evaluation. Consequently for a given patient follow-up controls become possible.

For a quantitative evaluation the absorptions of the standards and controls are graphically drawn *point-to-point* 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. As curve fit *point-to-point* has to be chosen. **The sample dilution factor (1:100) must not be considered in the calculation. It is already contained in the concentration declaration of the standards.**

10. ASSAY CHARACTERISTICS

Diphtheria Toxoid ELISA	IgG
Intra-Assay-Precision	7.5 %
Inter-Assay-Precision	4.9 %
Inter-Lot-Precision	2.3 – 7.4 %
Analytical Sensitivity	0.004 IU/mL
Recovery	96 – 102 %
Linearity	78 – 133 %
Cross-Reactivity	No cross-reactivity to Clostridium tetani
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
Clinical Specificity	94 %
Clinical Sensitivity	94 %
Measuring Range	0.01 – 1.0 IU/mL







Manufactured for :

Immuno-Biological Laboratories, Inc. (IBL-America)
 8201 Central Ave. NE, Suite P, Minneapolis, Minnesota 55432, USA
 Phone: +1 (763) - 780-2955 Fax.: +1 (763) - 780-2988
 Email: info@ibl-america.com Web: www.ibl-america.com

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SYMBOLS USED WITH IBL-AMERICA ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
	European Conformity	CE-Konfirmationskennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeitsdatum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità