Instructions for Use

Diphtheria IgG ELISA

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

REF **RE56191**

Σ **12x8**

2-8°C

EU: IVD CE

I B L I N T E R N A T I O N A L G M B H

Flughafenstrasse 52a
D-22335 Hamburg, Germany

Phone: +49 (0)40-53 28 91-0
Fax: +49 (0)40-53 28 91-11

IBL@IBL-International.com
www.IBL-International.com

1. INTENDED USE

Enzyme immunoassay for the quantitative determination of IgG class antibodies against Diphtheria in human serum and plasma.

2. SUMMARY AND EXPLANATION

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. The etiologic agent is the *Corynebacterium diphtheriae*. Its pathogenicity is based on the secretion of an exotoxin that is circulating in the blood and affecting the heart muscle, kidneys and CNS. Only the toxigenic strains are pathogenic.
Depending on the stage of disease, the three types 'slight, middle and serious' can be distinguished. The grade of disease depends on the immune status of the child. Usually, limited Diphtheria arises, whereas in case of an immune suppression, severe Diphtheria is observed. As a result of this course of disease, the patient 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. TEST PRINCIPLE

Solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The wells are coated with antigen. Specific antibodies of the sample binding to the antigen coated wells are detected by a secondary enzyme conjugated antibody (E-Ab) specific for human IgG. After the substrate reaction the intensity of the color developed is proportional to the amount of IgG-specific antibodies detected. Results of samples can be determined directly using the standard curve.

4. WARNINGS AND PRECAUTIONS

1. For *in-vitro diagnostic* use only. For professional use only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. In case of severe damage of the kit package please contact IBL or your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
4. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
6. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details. Material Safety Data Sheets for this product are available on the IBL-Homepage or upon request directly from IBL.
7. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
8. The cleaning staff should be guided by the professionals regarding potential hazards and handling.
9. Avoid contact with Stop solution. It may cause skin irritations and burns.
10. Some reagents contain sodium azide (NaN_3) as preservatives. In case of contact with eyes or skin, flush immediately with water. NaN_3 may react with lead and copper plumbing to form explosive metal azides. When disposing reagents, flush with a large volume of water to avoid azide build-up.
11. All reagents of this kit containing human serum or plasma have been tested and were found negative for anti-HIV I/II, HBsAg and anti-HCV. However, a presence of these or other infectious agents cannot be excluded absolutely. For this reason reagents should be treated as potential biohazards in use and for disposal.

5. STORAGE AND STABILITY

The kit is shipped at ambient temperature and should be stored at 2-8 °C. Keep away from heat or direct sunlight. The storage and stability of specimens and prepared reagents is stated in the corresponding chapters.

The unopened reagents are stable until the expiry date indicated. The Kit is stable up to 3 months after the first opening when the Microtiterplate is packed in a tightly closed bag, the bottles are closed with their screw caps and the kit is stored at 2-8°C.

6. SPECIMEN COLLECTION AND STORAGE

Serum, Plasma (EDTA, Heparin)

The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Do not use grossly hemolytic, icteric or grossly lipemic specimens. Samples appearing turbid should be centrifuged before testing to remove any particulate material.

Storage:	2-8 °C	-20 °C	Keep away from heat or direct sunlight. Avoid repeated freeze-thaw cycles.
Stability:	7 days	> 7 days	

7. MATERIALS SUPPLIED

Quantity	Symbol	Component
1 x 12 x 8	MTP	**Microtiter Plate** Break apart strips. Coated with specific antigen.
1 x 5 x 2 mL	CAL A-E	**Standards A-E** 0; 0.01; 0.1; 0.5; 1.0 IU/mL Ready to use. Calibrated against WHO 00/496. Contains: Human serum, IgG antibodies against Diphtheria, PBS, 0.01% Methylisothiazolinone and 0.01 % Bromonitrodioxane.
1 x 15 mL	ENZCONJ IgG	**Enzyme Conjugate IgG** Red colored. Ready to use. Contains: anti-human IgG, conjugated to peroxidase (rabbit), protein-containing buffer, 0.01 % Methylisothiazolinone, 0.01 % Bromonitrodioxane and 5 mg/L ProClin.
1 x 60 mL	DILBUF	**Diluent Buffer** Ready to use. Contains: PBS Buffer, BSA, < 0.1 % NaN_3.
1 x 60 mL	WASHBUF CONC	**Wash Buffer,** Concentrate (10x) Contains: PBS Buffer, Tween 20.
1 x 15 mL	TMB SUBS	**TMB Substrate Solution** Ready to use. Contains: TMB.
1 x 15 mL	TMB STOP	**TMB Stop Solution** Ready to use. 0.5 M H_2SO_4.
2 x	FOIL	**Adhesive Foil** For covering of Microtiter Plate during incubation.
1 x	BAG	**Plastic Bag** Resealable. For dry storage of non-used strips.

8. MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettes (Multipette Eppendorf or similar devices, < 3 % CV). Volumes: 5; 50; 100; 500 µL
2. Calibrated measures
3. Tubes (1 mL) for sample dilution
4. 8-Channel Micropipettor with reagent reservoirs
5. Wash bottle, automated or semi-automated microtiter plate washing system
6. Microtiter plate reader capable of reading absorbance at 450 nm (reference wavelength 600-650 nm)
7. Bidistilled or deionised water
8. Paper towels, pipette tips and timer

9. PROCEDURE NOTES

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18-25 °C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each component and specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.
4. Use a pipetting scheme to verify an appropriate plate layout.
5. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.
6. Microtiter plate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microtiter plate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells.
7. Humidity affects the coated wells/tubes. Do not open the pouch until it reaches room temperature. Unused wells/tubes should be returned immediately to the resealed pouch including the desiccant.

10. PRE-TEST SETUP INSTRUCTIONS

10.1. Preparation of Components

⚠	The contents of the kit for 96 determinations can be divided into 3 separate runs. The volumes stated below are for one run with 4 strips (32 determinations).

Dilute / dissolve	Component		Diluent	Relation	Remarks	Storage	Stability
20 mL	**WASHBUF** **CONC**	180 mL	bidist. water	1:10	Warm up at 37°C to dissolve crystals, if necessary. Mix vigorously.	2-8 °C	8 weeks

10.2. Dilution of Samples

Sample	to be diluted	with	Relation	Remarks
Serum / Plasma	generally	**DILBUF**	1:101	e.g. 5 µL + 500 µL **DILBUF**

Samples containing concentrations higher than the highest standard have to be diluted further.

11. TEST PROCEDURE

1.	Pipette **100 µL** of each **Standard and diluted serum or plasma sample** into the respective wells.
2.	Cover plate with adhesive foil. **Incubate 60 min** at **18-25 °C.**
3.	Remove adhesive foil. Discard incubation solution. Wash plate **3 x** with **300 µL** of **diluted Wash Buffer.** Remove excess solution by tapping the inverted plate on a paper towel.
4.	Pipette **100 µL** of **Enzyme Conjugate** into each well.
5.	Cover plate with new adhesive foil. **Incubate 30 min** at **18-25 °C.**
6.	Remove adhesive foil. Discard incubation solution. Wash plate **3 x** with **300 µL** of **diluted Wash Buffer.** Remove excess solution by tapping the inverted plate on a paper towel.
7.	For adding of Substrate and Stop Solution use, if available, an 8-channel Micropipettor. Pipetting should be carried out in the same time intervals for Substrate and Stop Solution. Use positive displacement and avoid formation of air bubbles.
8.	Pipette **100 µL** of **TMB Substrate Solution** into each well.
9.	**Incubate 20 min** at **18-25 °C** in the dark (without adhesive foil).
10.	Stop the substrate reaction by adding **100 µL** of **TMB Stop Solution** into each well. Briefly mix contents by gently shaking the plate. Color changes from blue to yellow.
11.	**Measure** optical density with a photometer at **450 nm** (Reference-wavelength: 600-650 nm) within **60 min** after pipetting of the Stop Solution.

12. QUALITY CONTROL

The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable standards/laws. All standards must be found within the acceptable ranges as stated on the QC Certificate. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls. It is recommended to participate at appropriate quality assessment trials.

In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

13. CALCULATION OF RESULTS

The obtained OD of the standards (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline or point-to-point curve, because these methods give the highest accuracy in the data calculation.

For the calculation of the standard curve, apply each signal of the standards (one obvious outlier of duplicates might be omitted and the more plausible single value might be used).

The concentration of the samples can be read directly from the standard curve.

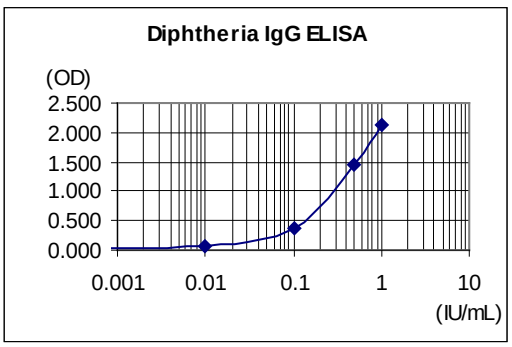
The initial dilution has been taken into consideration when reading the results from the graph. Results of samples of higher predilution have to be multiplied with the dilution factor.

Samples showing concentrations above the highest standard have to be diluted as described in PRE-TEST SETUP INSTRUCTIONS and reassayed.

Typical Calibration Curve

(Example. Do not use for calculation!)

Standard	IU/mL	OD_{Mean}
A	0	0.021
B	0.01	0.056
C	0.1	0.360
D	0.5	1.464
E	1.0	2.133



14. INTERPRETATION OF RESULTS

IU/mL	Interpretation
< 0.1 IU/mL	basic immunisation recommended
0.1 - 1.0 IU/mL	booster vaccination recommended
1.0 - 1.5 IU/mL	to be boosted in 5 y
1.5 - 2.0 IU/mL	to be boosted in 7 y
> 2.0 IU/mL	to be boosted in 10 y

The results themselves should not be the only reason for any therapeutical consequences. They have to be correlated to other clinical observations and diagnostic tests.

15. EXPECTED VALUES

In an in-house study, apparently healthy subjects showed the following results:

Range	x/56	%
< 0.01 IU/mL	0	0
0.01 – 0.1 IU/mL	0	0
0.1 – 1.0 IU/mL	40	71
> 1.0 IU/mL	16	29

It is recommended that each laboratory establishes its own range of normal values.

16. LIMITATIONS OF THE PROCEDURE

Specimen collection and storage have a significant effect on the test results. See SPECIMEN COLLECTION AND STORAGE for details.

For cross-reactivities, see PERFORMANCE.

Azide and thimerosal at concentrations > 0.1 % interfere in this assay and may lead to false results.

The following blood components do not have a significant effect (+/- 20% of expected) on the test results up to the below stated concentrations:

Hemoglobin	8.0 mg/mL
Bilirubin	0.3 mg/mL
Triglyceride	5.0 mg/mL

17. PERFORMANCE

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-reactivities were found to: Clostridium tetani
Clinical specificity	94 %
Clinical sensitivity	94 %
Measuring range	0.01 – 1.0 IU/mL

18. PRODUCT LITERATURE REFERENCES

1. Björkholm, B, Granström M, Hagberg L, Diphtheria antitoxin titres six years after immunization of adults, Vaccine 14 (17/18): 1633-36 (1996)
2. CDC, Diphtheria Epidemic – New Independent States of the Former Soviet Union, 1990-1994; MMWR 44: 177 (1995)
3. Choi JH, Choo EJ, Huh A, Choi SM, Eom JS, Lee JS, Park SH, Kang JH, Immunogenicity and safety of diphtheria-tetanus vaccine in adults, J Korean Med Sci 25(12): 1727-32 (2010)
4. Diphtheria Outbreak – Russian Federation 1990–1993, Morb Mortal Wkly Rep 42 (43): 840 (1993)
5. Engelhard R, Diphtherie-Nachweis und Abgrenzung zu anderen Korynebakterien, Med. Mikrobiologie 12: 628-632 (1997)
6. Epidemiologisches Bulletin 02/2000, Impfempfehlungen der Ständigen Impfkommission (STIKO) am Robert Koch-Institut, Januar 2000
7. Gupta RK, Higham, S, Gupta CK, Rost B, Diphtheria antitoxin levels in blood and plasma donors, J Infect Dis 173: 1493-7 (1996)
8. Hasselhorn HM, Nübling M, Tiller FW, Hofmann F, Diphtherie-Auffrischung bei Erwachsenen, Dtsch Med Wschr 122: 281-286 (1997)
9. Kang JH, Hur JK, Kim JH, Lee KI, Park SE, Ma SH, Lee MS, Baek SY, Hong SH, Min HK, Age Related Seroepidemiological Study of Diphtheria among Koreans, Korean J Infect Dis 32(1): 1-7 (2000)
10. Kjeldsen, K, Simonsen O, Heron I, Immunity against diphtheria and tetanus in the age group 30-70 years, Scand J Infect Dis 20: 177-85 (1988)
11. Kjeldsen K, Simonsen O, Immunity against diphtheria 25-30 years after primary vaccination in childhood, Lancet 1: 900-2 (1985)
12. Kuhlmann WD, Rieger J, Diphtheria Immunity of a West German population by measurement of antitoxin antibodies with Enzyme-Linked-Immunosorbent Assay, Immunol Infect Dis 5: 10-14 (1995)
13. Naumann P, Weber HG, Diphtherie-Immunität bei Schulanfängern und nach Wiederimpfung mit d-Impfstoff, Dtsch med Wschr 117: 1308-12 (1992)
14. Pietsch M, Impfserologie zur Ergänzung von Impfungen, Der Allgemeinarzt 18: 1155-56 (1993)
15. Pilars de Pilar CE, Spiess H, Diphtherie- und Tetanusantikörper bei Kindern und jungen Erwachsenen, Dtsch med Wschr 106: 1341-45 (1981)
16. Rieger J, Kuhlmann D, Diphtherieimmunität der Bevölkerung in Deutschland, Gesundheitswesen 56: 667-71 (1994)
17. Saxena S, Jais M, Dutta R, Dutta AK, Serological immunity to diphtheria and tetanus in healthy adults in Delhi, India, Trop Doct 39(3): 160-3 (2009)
18. Speranza FA, Ishii SK, Hirata R Jr, Mattos-Guaraldi AL, Milagres LG, Diphtheria toxin IgG levels in military and civilian blood donors in Rio de Janeiro, Brazil, Braz J Med Biol Res. 43(1): 120-3 (2010)
19. Zarei S, Jeddi-Tehrani M, Akhondi MM, Zeraati H, Pourheidari F, Ostadkarampour M, Tavangar B, Shokri F, Primary immunization with a triple diphtheria-tetanus-whole cell pertussis vaccine in Iranian infants: an analysis of antibody response**,** Iran J Allergy Asthma Immunol 8(2): 85-93 (2009)

Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

REF	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.: / Αριθμός-Κατ.:
LOT	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
CONC	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
LYO	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο
IVD	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluació. / Kit de avaliação. / Kit di evaluazione. / Κιτ Αξιολόγησης.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.
	Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!

Symbols of the kit components see MATERIALS SUPPLIED.
Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.
Voir MATERIEL FOURNI pour les symbôles des composants du kit.
Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.
Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.
Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.
Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

COMPLAINTS: Complaints may be submitted initially written or vocal. Subsequently they need to be filed including the test performance and results in writing in case of analytical reasons.

WARRANTY: The product is warranted to be free from material defects within the specific shelf life and to comply with product specifications delivered with the product. The product must be used according to the Intended use, all instructions given in the instructions for use and within the product specific shelf life. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement.

LIMITATION OF LIABILITY: IN ALL CIRCUMSTANCES THE EXTENT OF MANUFACTURER'S LIABILITY IS LIMITED TO THE PURCHASE PRICE OF THE KIT(S) IN QUESTION. IN NO EVENT SHALL MANUFACTURER BE LIABLE FOR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING DAMAGES FOR LOST PROFITS, LOST SALES, INJURY TO PERSON OR PROPERTY OR ANY OTHER INCIDENTAL OR CONSEQUENTIAL LOSS.


IBL International GmbH
Flughafenstr. 52A, 22335 Hamburg, Germany

Tel.: + 49 (0) 40 532891 -0 Fax: -11
E-MAIL: IBL@IBL-International.com
WEB: http://www.IBL-International.com