Instructions for Use

Haemophilus influenzae B IgG ELISA

Enzyme immunoassay for determination of IgG antibodies against polyribosylribitolphosphate of Haemophilus influenzae type B in human serum and plasma.

REF **RE56351**

Σ **96**

 **2-8°C**

EU: IVD CE

Republic of India: *For research use only. Not for use in diagnostic procedures.*

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 determination of IgG antibodies against polyribosylribitolphosphate (PRP) of *Haemophilus influenzae* type B in human serum and plasma.

2. SUMMARY AND EXPLANATION

Haemophilus influenzae type B (HiB) is a very common cause of invasive critical infectious diseases in children up to the age of six. Following infection the symptoms of the disease include: Pericarditis, osteomyelitis, meningitis, encephalitis, pneumonia, sinusitis and otitis. In many cases the disease is lethal or leads to neurological damage, which cannot always be prevented by rapid antibiotic therapy. The underlying reason for the disease is very often a latent immunodeficiency with a specifically reduced humoral immune response to the polyribosylribitolphosphate (PRP) in the polysaccharide encapsulation of the bacterium. In children another reason is the immaturity of the immune system. Today often the term "immunocompromised patients" is used, comprising all acquired and innate specific and unspecific immunodeficiencies.
As a result, in children of 3 months of age or older a vaccination with different sorts of PRP-containing vaccines is recommended. This can lead to a clear reduction in the number of infections with *Haemophilus influenzae* type B.
The titer of antibodies produced by vaccination can be used to confirm whether the vaccination has been successful. The HiB IgG is used to measure the level of PRP-specific IgG-antibodies following a 4-6 week period after complete immunization to monitor the humoral immune status of children or other individuals at risk.
Monitoring of the humoral immunostatus after vaccination. Verification of the diagnosis *Haemophilus influenzae* type B infection by repeated monitoring of antibody concentrations. Risk assessment in immunocompromised patients leading to a failure of vaccination with a PRP-containing vaccine.

This group comprises:
Children under 2 years having had an infection with *Haemophilus influenzae* type B,
Children with chronic, recurring bacterial infections of the respiratory tract,
Children with chronic otitis,
Patients with confirmed humoral immuno-deficiencies (IgG-2-deficiency, IgA-deficiency),
Patients with confirmed granulocyte deficiencies,
Patients under chemo or cytostatic therapy,
Children after splenectomy,
Patients with sickle-cell anaemia,
Patients with trisomy 21 (Down) syndrome, and certain ethnic groups.

3. TEST PRINCIPLE

The HiB-IgG is a two-step-ELISA. The wells in the ELISA test strips are coated with PRP. During incubation of diluted serum or plasma samples specific antibodies against bind to the solid phase (**sample incubation**). Following a washing procedure all unbound and non-specific components are washed away. During the second incubation step, the **conjugate reaction**, a peroxidase-conjugated anti-human IgG-antibody (anti-human-IgG-HRP) labels the previously specifically bound IgG. In a second washing procedure unbound conjugate is removed. In a third incubation step the **substrate reaction** takes place. The peroxidase part of the bound conjugate oxidizes tetramethylbenzidine (TMB) to a blue substance. This reaction is stopped by adding sulfuric acid and the colour changes to yellow. The colour intensity is directly proportional upon the concentration of the PRP-specific antibodies. The absorbance is measured with an ELISA reader at 450 nm. The antibody concentration in the sample can be determined using a reference 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. 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 microtiter strips are stable **up to 6 months in the broken**, but tightly closed bag when stored at 2–8°C.

6. SPECIMEN COLLECTION AND STORAGE

Human serum, Citrate-, EDTA- or Heparin-Plasma.

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 (Aliquots)	Keep away from heat or direct sunlight. Avoid repeated freeze-thaw cycles.
Stability:	6 weeks	6 months	

7. MATERIALS SUPPLIED

Quantity	Symbol	Component
1 x 12 x 8	**MTP**	**Microtiter Plate** Coated with PRP from *Haemophilus influenzae Typ B*. Ready to use!
1 x 0.3 mL	**ENZCONJ** **CONC**	**Enzyme Conjugate,** Concentrate Anti-human-IgG-peroxidase; colored blue. Dilute before use!
5 x 0.35 mL	**CAL 1-5**	**Standard 1-5,** Concentrate **(NISBC code 09/222)** Human sera with stabilizers and preservatives. **Concentrations are lot specific as indicated on the bottle labels.** Dilute before use!
2 x 0.35 mL	**POS LL,** **POS HL**	**Positive Control LL+HL,** Concentrate Positive control sera, LL, "Low Level", HL, "High Level"; for testing accuracy; human sera with stabilizers and preservatives. **Concentrations are lot specific as indicated on the bottle labels.** Dilute before use!
2 x 75 mL	**DILBUF**	**Incubation Buffer (Dilution Buffer)** 0.01 M Tris/HCl; pH 7.4; contains detergent; 0.01% (w/v) thimerosal; colored red. Ready to use!
1 x 100 mL	**WASHBUF** **CONC**	**Wash Buffer,** Concentrate (10x) Contains: phosphate buffer
1 x 15 mL	**TMB SUBS**	**TMB Substrate Solution** Substrate solution contains TMB (tetramethylbenzidine). Ready to use!
1 x 15 mL	**TMB STOP**	**TMB Stop Solution** Stop solution, 0.5 M sulphuric acid. Ready to use!
2 pieces	**FOIL**	**Adhesive Foil**

Notice: Wash buffer, substrate and stop solution can be exchanged with following products: TBE / FSME IgG (RE57401), TBE / FSME IgM (RE57411), Tetanus (RE57441) and Diphtherie (RE57431).

8. MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettes (Multipette Eppendorf or similar devices, < 3 % CV). Volume: 20; 100; 500; 1000 µL
2. Vortex mixer
3. Tubes 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. It is advised to determine samples in duplicate to be able to identify potential pipetting errors.
5. Use a pipetting scheme to verify an appropriate plate layout.
6. 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.
7. 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.
8. 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	with	Diluent	Relation	Remarks	Storage	Stability
60 µL	**ENZCONJ** **CONC**	6 mL	**DILBUF**	1:101	Mix carefully.	2-8°C	1 hour
30 mL	**WASHBUF** **CONC**	270 mL	**Aqua dest**.	1:10	Mix carefully.	2-8°C	8 weeks

10.2. Dilution of Standards, Controls and Samples

	to be diluted	with	Relation	Remarks
CAL 1-5 **CONTROL LL** **CONTROL HL**	generally	**DILBUF**	1:26	e.g. 20 µL + 500 µL
Serum / Plasma	generally	**DILBUF**	1:26	e.g. 20 µL + 500 µL

For the determination by standard curve calibrators 1-5 and control sera are needed.

11. TEST PROCEDURE

1.	Pipette **100 µL** of **diluted Calibrator, diluted Controls and diluted sample** into the respective wells of the Microtiter Plate.
2.	Cover plate with adhesive foil. **Incubate 1 h** at **RT (18-25°C).**
3.	Remove adhesive foil. Discard incubation solution. Wash plate **3 x** with **250 µL** of diluted **Wash Buffer.** Remove excess solution by tapping the inverted plate on a paper towel.
4.	Pipette **100 µL** of diluted **Enzyme Conjugate** into each well.
5.	Cover plate with adhesive foil. **Incubate 1 h** at **RT (18-25°C).**
6.	Remove adhesive foil. Discard incubation solution. Wash plate **3 x** with **250 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 30 min** at **RT (18-25°C).**
10.	Stop the substrate reaction by adding **100 µL** of **TMB Stop Solution** into each well. Briefly mix contents by gently shaking the plate.
11.	**Measure** optical density with a photometer at **450 nm ± 10 nm** (Reference-wavelength: 600-650 nm) within **10 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 comparable standards/laws. User and/or laboratory must have a validated system to get diagnosis according to GLP. All kit controls must be found within the acceptable ranges as stated on the labels and 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.

x-axis (log): Concentration in [µg/mL]

y-axis (lin): Absorbance (optical density)

A good fit is provided with cubic spline, 4 Parameter Logisitcs or Logit-Log. (E.g. 4-Parameter equation 1)

Equation 1: $\mathbf{Y = d+(a-d)/(1+(x/c)^b)}$

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 from the standard curve.

Samples suspected to contain concentrations higher than the highest standard have to be diluted with Diluent Buffer. Measured results must be multiplied with the dilution factor to obtain corrected results. Concentration values obtained from citrated plasma must be multiplied by 1.1

Validation criteria:

See QC-certificate.

14. INTERPRETATION OF RESULTS

The determination of PRP-specific antibodies shows the level of humoral immune reaction after an immunization with a PRP-containing vaccine or a clinical apparent or inapparent infection with *Haemophilus influenzae* type B. When this test is negative and the patient belongs to a risk group (conf. "Indications"), it can be assumed that a risk for an infection with *Haemophilus influenzae* type B exists. Humoral immunoreactions after an infection with non-typeable *Haemophilus influenzae* are not detected by HiB IgG. The failure to seroconvert after vaccination is indicative of the ability to react to bacterial carbohydrate antigens. A polysaccharide-specific immunodeficiency can be observed in patients with chronic bronchitis, recurring pneumoniae, intrinsic bronchial asthma or bronchiectases of unclear genesis.

It has been shown in publications [2, 8, 9, 10, 11] that an **antibody concentration under 0.15 µg/mL** gives insufficient protection against *Haemophilus influenzae* type B.

Antibody concentrations between 0.15 and 1.0 µg/mL indicate that the patient has been immunised with PRP or had an infection with HiB.

But only **antibody concentrations over 1.0 µg/mL** represent a sufficient natural immunity or an aquired protection after the third vaccination.

15. PERFORMANCE

Intraassay variation:

2 samples in the concentration range of the calibrators measured 20 times each with one lot in double determination. The intraassay variation was ≤10%.

Interassay variation:

POS HL and POS LL controls measured in 20 runs on different days with one lot in double determination. The interassay precision was between 9 and 12 % respectively.

Lower detection limit: ≤ 0.1 µg/mL (lot-specific).

16. PRODUCT LITERATURE REFERENCES

1. Barra, A. et al.: Measurement of Anti-*Haemophilus influenzae* type B capsular polysaccharide antibodies by ELISA. J. Immunol. Meth. 115, 111 (1988)
2. Claesson, BA. et al.: Development of serum antibodies of the immuno-globulin G class and subclasses against the capsular polysaccharide of *Haemophilus influenzae* type B in children and adults with invasive infections. J. Clin. Microbiol. 26, 2549 (1988)
3. Dolan, K.T. et al.: An enzyme-linked immunosorbent assay for quantitation of *Haemophilus influenzae* type B polysaccharide-specific IgG1 and IgG2 in human and infant rhesus monkey sera. J. Immunoassay 12, 543 (1991)
4. Hetherington, S.V. et al.: Correlation between antibody affinity and serum bactericidal activity in infants. J. Infect. Dis. 165, 753 (1992)
5. Isaacs, D.: Infectious diseases. Editorial overview. Curr. Opin. Pediat. 4, 45 (1992)
6. Kristensen, K., Weis Bentzon, M.: Relation between enzyme-linked immunosorbent assay and radioimmunoassay for the detection of antibodies to the capsular polysaccharide of *Haemophilus influenzae* type B. Acta. path. microbiol. immunol. scand. Sect. C 100, 142 (1992)
7. Lagergard, T. et al.: Comparison between radioimmunoassay and direct and indirect enzymelinked immunosorbent assay for determination of antibodies against *Haemophilus influenzae* type B capsular polysaccharide. J. Clin. Microbiol. 26, 2554 (1988)
8. Käyhty, H. et al.: Antibodies response to capsular polysaccharides of groups A and C *Neisseria meningitis* and *Haemophilus influenzae* type b during bacteremic disease. Infect. Dis. 143, 32 (1981)
9. Anderson, P.: The protective level of serum antibodies to the capsular polysaccharide of *Haemophilus influenzae* type B. J. Infect. Dis. 149, 1034 (1984)
10. Zielen, S. et al.: Untersuchung zur Effektivität der *Haemophilus-influenzae*-B-Diphtherie-Konjugatimpfung bei deutschen Kindern. Monatsschr. Kinderheilkunde 140, 852 (1992)
11. Zielen, S., Ahrens, P.: *Haemophilus influenzae* Typ B-Impfserologie. Ellipse 11, 15 (1994)
12. Togni, G. u. a.: Präanalytik. Schweiz. Med. Forum. 6, 113-120 (2002)

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.

IBL AFFILIATES WORLDWIDE

IBL International GmbH Flughafenstr. 52A, 22335 Hamburg, Germany	Tel.: E-MAIL: WEB:	+ 49 (0) 40 532891 -0 Fax: -11 IBL@IBL-International.com http://www.IBL-International.com
IBL International Corp. 194 Wildcat Road, Toronto, Ontario M3J 2N5, Canada	Tel.: E-MAIL: WEB:	+1 (416) 645 -1703 Fax: -1704 Sales@IBL-International.com http://www.IBL-International.com

Symbols Version 3.5 / 2012-01-20