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**User's Manual**

# Human Haemophilus Influenza B IgM ELISA Kit

REF **DEIA1650M**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.


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# PRODUCT INFORMATION

## Intended Use

Human Haemophilus Influenza B IgM ELISA is an enzyme immunoassay for determination of IgM antibodies against polyribosylribitol phosphate of Haemophilus influenzae type B in human serum and plasma.

## Principles of Testing

The HiB-IgM 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 IgM-antibody (anti-human-IgM-HRP) labels the previously specifically bound IgM. 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.

## Reagents And Materials Provided

1. Coated assay plate: 1(96 wells)
2. Negative Control: 1 x 800 µl
3. Positive Control: 1 x 800 µl
4. HRP-conjugate (100 x concentrate): 1 x 120 µl
5. HRP-conjugate Diluent: 1 x 20 ml
6. Sample Diluent: 2 x 20 ml
7. Wash Buffer (20 x concentrate): 1 x 40 ml
8. TMB Substrate: 2 x 6 ml
9. Stop Solution: 1 x 10 ml
10. Adhesive Strip (For 96 wells): 3
11. Instruction manual: 1

## 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

## Storage

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

The microtiter strips are stable up to 6 mon in the broken, but tightly closed bag when stored at 2-8°C.

## Specimen Collection And Preparation

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. Stability: 2-8°C for 6 weeks, and ≤ -20°C (Aliquots) for 6 months.

Keep away from heat or direct sun light. Avoid repeated freeze-thaw cycles.

## Reagent Preparation

* Kindly use graduated containers to prepare the reagent. Please don't prepare the reagent directly in the Diluent vials provided in the kit.

* Bring all reagents to room temperature (18-25°C) before use for 30min.

* Distilled water is recommended to be used to make the preparation for reagents. Contaminated water or container for reagent preparation will influence the detection result.

**HRP-conjugate (1x)** - Centrifuge the vial before opening.

HRP-conjugate requires a 100-fold dilution. A suggested 100-fold dilution is 10 µl of HRP-conjugate + 990 µl of HRP-conjugate Diluent.

**Wash Buffer(1x)**- If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved.

Dilute 25 ml of Wash Buffer Concentrate (20x) into deionized or distilled water to prepare 500 ml of Wash Buffer (1x).

## Assay Procedure

**Procedure Note:**

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. Microplate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microplate 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.

**Procedure:**

1. Add 100µl of Negative Control, Positive Control or diluted Sample per well. Samples and controls must be assayed in duplicate. Cover with the adhesive strip provided. Incubate 1 h at RT (22-28°C).
2. Remove adhesive foil. Discard incubation solution. Wash plate 5 x with 300 µL of diluted Wash Buffer. Remove excess solution by tapping the inverted plate on a paper towel.
3. Pipette 100 µL of diluted Enzyme Conjugate into each well. Cover plate with adhesive foil. Incubate 1 h at RT (22-28°C).
4. Remove adhesive foil. Discard incubation solution. Wash plate 5 x with 300 µL of diluted Wash Buffer. Remove excess solution by tapping the inverted plate on a paper towel.
5. 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.
6. Pipette 100 µL of TMB Substrate Solution into each well. Incubate 30 min at RT (22-28°C).
7. Stop the substrate reaction by adding 100 µL of Stop Solution into each well. Briefly mix contents by gently shaking the plate.
8. Measure optical density with a photometer at 450 nm (Reference-wavelength: 600-650 nm) within 10 min after pipetting of the Stop Solution.

## Interpretation Of Results

For calculation HiB-IgM, compare the sample well with control.

* While $OD_{sample}$/ $OD_{negative} \geq 2.1$: Positive

* While $OD_{sample}$/ $OD_{negative} < 2.1$: Negative

## Reproducibility

Intra-assay variation: ≤ 10%

Inter-assay variation: ≤ 15%

## Precautions

1. 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.
2. In case of severe damage of the kit package please contact CD 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.
3. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
4. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
5. Reagents of this kit containing hazardous material may cause eye and skin irritations. Material Safety Data Sheets for this product are available on the CD-Homepage or upon request directly from CD.
6. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
7. Avoid contact with Stop solution. It may cause skin irritations and burns.
8. 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 and therefore reagents should be treated as potential biohazards in use and for disposal.