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User´s Manual

Mycobacterium tuberculosis IgM ELISA

Enzyme immunoassay for the determination of human IgM antibodies against Mycobacterium tuberculosis in serum and plasma

REF IB79285

96 wells

RUO **For Research Use Only – Not for Use in Diagnostic Procedures**

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1. INTENDED USE

The Mycobacterium tuberculosis IgM antibody ELISA kit has been designed for the determination of specific IgM antibodies against Mycobacterium tuberculosis in serum and plasma. For research use only, not for use in diagnostic procedures.

2. PRINCIPLE OF THE TESTS

The Mycobacterium tuberculosis IgM antibody test kit is based on the principle of the enzyme immunoassay (EIA). Mycobacterium tuberculosis antigen is bound on the surface of the microtiter strips. Diluted serum or ready-to-use standards are pipetted into the wells of the microtiter plate. A binding between the IgM antibodies of the serum and the immobilized Mycobacterium tuberculosis 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-IgM 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 IgM antibodies is directly proportional to the intensity of the color.

3. LIMITATIONS, PRECAUTIONS AND GENERAL COMMENTS

- Only for research 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.

4. REAGENTS PROVIDED

Symbol	Components	Volume / Qty.
SORB MT	Mycobacterium tuberculosis antigen coated microtiter strips	12
CAL A	Calibrator A (Negative Control)	2 mL
CAL B	Calibrator B (Cut-Off Standard)	2 mL
CAL C	Calibrator C (Weak Positive Control)	2 mL
CAL D	Calibrator D (Positive Control)	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 (10×)	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.

4.1. Microtiter Strips
12 strips with 8 breakable wells each, coated with M. tuberculosis antigen mixture (recombinant Mycobacterium tuberculosis antigens, with 18, 36 and 40 kDa). Ready-to-use.

4.2. Calibrator A (Negative Control)
2 mL, protein solution diluted with PBS, contains no IgM antibodies against Mycobacterium tuberculosis. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

4.3. Calibrator B (Cut-Off Standard)
2 mL human serum diluted with PBS, contains a low concentration of IgM antibodies against Mycobacterium tuberculosis. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

4.4. Calibrator C (Weak Positive Control)
2 mL, human serum diluted with PBS, contains a medium concentration of IgM antibodies against Mycobacterium tuberculosis. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

4.5. Calibrator D (Positive Control)
2 mL, human serum diluted with PBS, contains a high concentration of IgM antibodies against Mycobacterium tuberculosis. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

4.6. Enzyme Conjugate
15 mL, anti-human-IgM-HRP (rabbit), in protein-containing buffer solution. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane and 5 mg/L Proclin™. Ready-to-use.

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

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

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

4.10. 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.

5. 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
- Reusable Plastic Bag for the dry storage of non-used strips

6. 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).

7. ASSAY PROCEDURE

7.1. 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.

7.2. 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.

8. EVALUATION

8.1. Evaluation (Cut-off)

The calculated absorptions for the sample, as mentioned above, are compared with the value for the cut-off standard. If the value of the sample is higher, there is a positive result. For a value below the cut-off standard, there is a negative result. It seems reasonable to define a range of +/-20 % around the value of the cut-off as a grey zone. In such a case the repetition of the test with the same serum or with a new sample of the same subject, taken after 2-4 weeks, is recommended. Both samples should be measured in parallel in the same run.
The positive control must show at least the double absorption compared with the cut-off standard.

8.2. Evaluation (U/mL)

The ready-to-use standards and controls of the Mycobacterium tuberculosis IgM antibody kit are defined and expressed in arbitrary units (U/mL). Consequently for a given subject follow-up controls become possible. The values for controls and standards in units are printed on the QC data sheet.
For this 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 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.
Calibrator B with its concentration of 10 U/mL serves as cut-off standard. Analogous to the cut-off evaluation a range of +/-20% around the cut-off is defined as a grey zone. Thus results between 8 and 12 U/mL are reported as borderline.
For doubtful IgM positive results and for the confirmation of positive reactions the absorption of Rheumatoid Factor should be conducted with an appropriate reagent (Cat. No. IB79000, RF-Adsorbent).

9. ASSAY CHARACTERISTICS

Mycobacterium ELISA	IgM
Intra-Assay-Precision	7.9 %
Inter-Assay-Precision	7.4 %
Inter-Lot-Precision	5.7 – 8.9 %
Analytical Sensitivity	1.22 U/mL
Recovery	87 – 91 %
Linearity	78 – 118 %
Cross-Reactivity	No cross-reactivity to Helicobacter pylori and Bordetella pertussis.
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
Specificity	100 %
Sensitivity	100 %

Manufactured for:
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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-Konfirmitäts-kennzeichnung	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	Mindesthaltbarkeits-datum	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