

TUB IgM ELISA HUMAN TUBERCULOSIS TEST

Microtiter plate based Enzyme assay for the quantitative detection of human IgM antibodies to *Mycobacterium tuberculosis* in serum or plasma. Intended for professional *in-vitro* diagnostic use.



REF: LIO-TUB03

Rev. 6.0 / 190625

INTENDED USE

The **TUB IgM ELISA** is an *in-vitro* diagnostic microtiter plate based Enzyme assay for the quantitative detection of human IgM antibodies to *Mycobacterium tuberculosis* in serum or plasma within 100 minutes.

This test is intended to support the diagnosis of **active tuberculosis (TB)** and is not suitable for contact screening of populations.

INTRODUCTION / FIELD OF APPLICATION

Human tuberculosis (TB) has become a global disease with its re-emergence in the Western countries in the last decades. According to WHO, more than 30 % of the world's population is estimated to be infected with the TB bacterium, *Mycobacterium tuberculosis*. TB is predominantly a disease of the respiratory tract, but can also affect other organs. People who are suffering from active pulmonary tuberculosis are highly infectious. TB kills yearly about 2 million people.

Although, the TB bacterium was identified more than 100 years ago, the diagnostic methods, which are currently available, suffer from high price, poor sensitivity and specificity and are time consuming. The diagnosis of TB is usually made based on a combination of several laboratory tests.

The **TUB IgM ELISA** is suitable for the quantitative detection of human IgM antibodies to *Mycobacterium tuberculosis* within 100 minutes. This test is an **additional tool** for rapid diagnosis of **active tuberculosis (TB)** using small amount of clinical sample.

PRINCIPLE OF THE TEST

The **TUB IgM ELISA** is based on the principle of the enzyme immunoassay (EIA) and is intended for the quantitative detection of human IgM antibodies to *Mycobacterium tuberculosis* in serum or plasma within 100 minutes.

Highly purified specific antigens are bound on the surface of the microtiter plate. Diluted serum or plasma from human are pipetted into the wells of the microtiter plate at once with the ready-to-use standard solutions. A binding between the antibodies of the samples and the immobilized antigens takes place. After 45 minute incubation at 37°C, the plate is rinsed with diluted wash buffer, in order to remove unbound material. Then conjugate solution (peroxidase-conjugated anti-human antibody) is added into the wells and incubated for 30 minutes at 37°C. In this step, the conjugate binds to the antigen-specific antibodies from the samples (if available) and standard solutions. After a further washing step, the substrate solution for the peroxidase is pipetted into the wells and incubated for 20 minutes at 37°C, inducing the development of a blue dye in the wells. The intensity of this reaction is dependent on the amount of specific antibodies in the samples. The colour development is terminated by the addition of a stop-solution, which changes the colour from blue to yellow. The resulting dye is measured by an ELISA reader at the wavelength of 450 nm (optionally 620 nm). The concentration of specific antibodies is directly proportional to the intensity of the colour.

SUPPLIED MATERIALS

Packaging sizes:

REF LIO-TUB03 (44 Tests):

1 microtiter plate (96 wells) with 12 x 8 breakable strips suitable for 44 samples (duplicate measurements), including liquids: sample diluent, wash buffer, 3 standard solutions, conjugate solution, substrate solution and stop-solution.

TEST COMPONENTS

	IVD	In-Vitro Diagnostic Medical Device
	PL	Microtiter plate (96 wells), ready-to-use, coated with <i>Mycobacterium tuberculosis</i> antigens (12 x 8 individual breakable strips) sealed in an aluminum pouch with a desiccant bag.
	PV	Sample Diluent: 100 mL, ready-to-use, in PE-vial, PBS/BSA buffer, contains 0.05% 5-bromo-5-nitro-1.3-dioxane.
	WP	Wash Buffer, 10 x concentrate: 60 mL, in PE-vial, PBS buffer with Tween 20, contains 0.05% 5-bromo-5-nitro-1.3-dioxane.
1	STA	3 standard solutions: 2 mL each, ready-to-use, in PE-vials, with different amounts of relevant specific antibodies against <i>Mycobacterium tuberculosis</i> , contains 0.05% 5-bromo-5-nitro-1.3-dioxane.
2		
3		
	CON	Anti-human-IgM-conjugate solution: 12 mL, ready-to-use, in PE-vial, contains 0.05% 5-bromo-5-nitro-1.3-dioxane.
	TMB	TMB substrate solution: 12 mL, ready-to-use, in PE-vial (black), contains a solution of tetramethylbenzidine (TMB).
	STO	Stop-solution: 12 mL, ready-to-use, in PE-vial, 0.2 M H ₂ SO ₄ .
	i	1 instructions for use.
		Plastic bag: Reseal-able, for the dry storage of unused microtiter plate/strips.

MATERIALS NEEDED BUT NOT SUPPLIED

- Stop watch.
- Containers for sample collection. We recommend using standard containers for blood collection.
- Microliter pipets and tips.
- ELISA Reader with filter (450 nm or optionally 620 nm).
- Automatic washer for microtiter plates (optional).
- Vials for sample preparation.
- Measuring cylinder for wash buffer preparation.
- De-ionized or distilled water.
- Incubator (37°C).

PREPARATION OF REAGENTS

WP Wash Buffer: Dilute 1:10 with de-ionized or distilled water before use (1 volume wash buffer + 9 volumes water). If crystals precipitate during the cold storage, the concentrate solution should be warmed up at 37°C for 15 minutes.

All other reagents are ready-to-use. No further preparation of reagents is necessary.

STABILITY AND STORAGE CONDITIONS

Store the test at 2 - 8°C. Unopened kit components are stable until the expiry date. The expiry date is printed on the labels of the aluminum pouch (microtiter plate), on the labels of the containers for the liquids and on the outer packaging.

Do not use if the aluminum pouch is damaged. **DO NOT FREEZE** or expose temperatures above 30°C (except wash buffer, if crystal precipitate occurs).

Aluminum pouch with microtiter plate: Keep the test in unopened aluminum bag at 2 - 8°C.

Opened aluminum pouch with microtiter plate: Use up microtiter plate within 6 months!

Liquids: Keep liquid components at 2 - 8°C. Unopened liquids are stable until the expiry date. After first opening the liquids are stable for 6 months, if the bottles are tightly closed after every usage.

WARNINGS AND PRECAUTIONS

- **ATTENTION:** Avoid contact of skin, eye and mucosa to the **STO** stop-solution. Causes severe skin burns and eye damage! Wear safety glasses, gloves and protective clothing!
 - **ATTENTION:** Avoid contact of skin, eye and mucosa to the **TMB** substrate solution. The solution may damage fertility or the unborn child! Wear safety glasses, gloves and protective clothing!
 - In accordance with Good Laboratory Practice (GLP) or ISO9001, all laboratory devices employed should be regularly checked for the accuracy and precision.
 - Use all reagents within the expiry period (printed on the labels).
 - Do not use reagents from different kit lots or batch codes and avoid mixing of reagents of different kit lots or batch codes.
 - Before use bring all reagents to room temperature (preferably 15-30°C)!
 - Only for human serum or plasma. Do not use the test with other body fluids.
 - Avoid contamination of the reagents. Do not use the same container for several samples!
 - Lipemic, haemolytic or bacterially contaminated samples should not be used.
 - Avoid the use of turbid samples, which may be contaminated with bacteria.
 - Avoid repeated freezing and thawing of the samples because it could lead to denaturation of the antibodies.
 - For *in-vitro* diagnostic use only! Do not ingest or swallow! Do not eat, drink and smoke in the laboratory! Do not work without wearing protective clothing (gloves, safety glasses and protective clothing)! Avoid the contact of kit reagents with skin, eye or mucosa.
 - All kit components should be considered as infectious agents. Decontaminate and dispose of residues of kit contents and samples in accordance to local regulations, e.g. by autoclaving or using a disinfecting solution.
 - Avoid contamination of the reagents by using separate disposable pipet tips. Close bottles tightly immediately after removing reagents.
 - Before pipetting, mix all reagents thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided.
 - Pipet with constant intervals, so that all wells of the microtiter plate have the same conditions!
 - For single use only. Do not use microtiter plate if the outer packaging (aluminum pouch) is damaged. After opening the pouch, the microtiter plate/strips must be used within 6 months. After opening store microtiter plate/strips in the plastic bag provided together with the desiccant bag.
- ## SAMPLE COLLECTION AND PREPARATION
- The **TUB IgM ELISA** test works best with fresh samples.
- Collection of whole blood from the vein:** Take the sample under standard laboratory conditions (aseptically, avoid haemolysis).
- Serum or plasma:** Separate the red blood cells as soon as possible (e.g. by centrifugation). If the test cannot be performed immediately after the sampling, the samples can be stored for up to 2 days (48 hours) at 2 - 8°C. For longer storage, the whole blood must be centrifuged (separate serum or plasma from red blood cells). Serum and plasma can be stored at temperatures below -20°C. Frozen samples must be thawed prior to testing and well mixed. Avoid repeated freezing and thawing of samples!



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TUB IgM ELISA HUMAN TUBERCULOSIS TEST

Microtiter plate based Enzyme assay for the quantitative detection of human IgM antibodies to *Mycobacterium tuberculosis* in serum or plasma. Intended for professional *in-vitro* diagnostic use.

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Sample preparation (serum or plasma): For the performance of the test, the samples (not the standard solutions) have to be diluted 1:200 with **PV** sample diluent (e.g. 5 µL sample + 995 µL sample diluent)!

ATTENTION: All standard solutions are ready-to-use and MUST NOT be diluted!

TEST PROCEDURE

Test procedure time is 100 minutes:

1. Preparation of reagents:

Equilibrate all kit components to room temperature (preferable 15 - 30°C). Dilute 10 x concentrated **WP** wash buffer 1:10 with de-ionized or distilled water (1 + 9 volume). Dilute serum or plasma samples 1:200 with **PV** sample diluent (e.g. 5 µL sample + 995 µL sample diluent)!

2. **STEP A:** Pipet 100 µL of 1:200 diluted samples, each ready-to-use **STA** standard solution and **PV** sample diluent for blank value into the wells (all in duplicates).

INCUBATION: 45 minutes at 37°C.

3. **STEP B:** Washing procedure: Empty the wells of the plate (dump or aspirate) and wash 3 x 300 µL per well with 1:10 diluted **WP** wash buffer.

Pipet 100 µL of **CON** conjugate solution into all wells.

INCUBATION: 30 minutes at 37°C.

4. **STEP C:** Repeat washing procedure (3 x 300 µL of 1:10 diluted **WP** wash buffer per well).

Pipet rapidly 100 µL of the **TMB** substrate solution into all wells.

INCUBATION: 20 minutes at 37°C in the dark.

5. **STEP D:** Terminate the substrate reaction: Pipet rapidly 100 µL of the **STO** stop-solution into all wells.

6. **Measuring procedure:** Measure the absorption at 450 nm (optional reference wavelength: 620 nm). The colour is stable for at least 60 minutes.

QUICK REFERENCE GUIDE OF TEST PROCEDURE

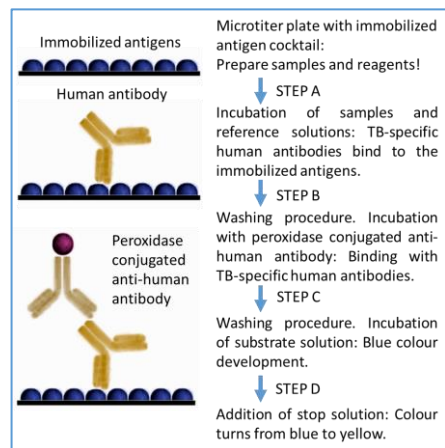


Fig. 1: Schematic presentation of test procedure of TUB IgM ELISA (REF: LIO-TUB03).

INTERPRETATION OF RESULTS

Calculate the **mean OD values** for the measured absorptions for every sample. Subtract the blank value from every calculated mean absorption. The difference between single values should not exceed 10% for the standard solutions and samples.

QUANTITATIVE RESULTS

The standard solutions of **TUB IgM ELISA** contain different amounts of relevant specific antibodies against *Mycobacterium tuberculosis* (TB antigens):

STA 1 Standard solution 1 contains no antibodies against *Mycobacterium tuberculosis*:

NEG negative control

STA 2 Standard solution 2 contains borderline amount of antibodies against *Mycobacterium tuberculosis*:

COC CUT-OFF control

STA 3 Standard solution 3 contains high amount of antibodies against *Mycobacterium tuberculosis*:

POS positive control

The mean OD of each sample can be compared with the OD values measured for the standard solutions. To reduce variations (day to day etc.), the measurements should be normalised. For this, divide all mean OD values for the sample by the average of standard solution 2 (COC):

$$\text{Normalised OD value (NV)} = \frac{\text{mean OD sample}}{\text{mean OD STA 2}}$$

The result of Human Tuberculosis Test kits (TUB ELISA) can be **NEGATIV**, **BORDERLINE** or **POSITIVE**.

ATTENTION: Diagnosis of TB should not be based only on the results of one assay. Take all clinical data (antibody titres, results of microscopy, culture, skin test e.g.) into consideration to reach the most accurate diagnostic conclusion.

CUT-OFF level

The CUT-OFF level of the **TUB IgM ELISA** is determined by the evaluation of several hundreds of samples from TB-patients and healthy individuals. But the CUT-OFF level may vary for patient populations from different regions and countries. Therefore, the CUT-OFF value should be determined individually by every new user. We recommend determining the CUT-OFF by measuring a panel of confirmed negative and positive cases (example is shown in figure 2).

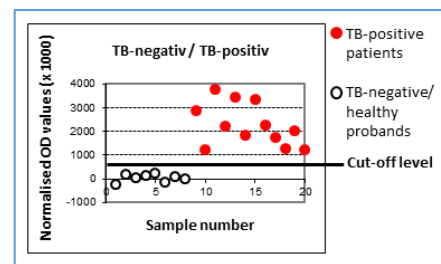


Fig. 2: Comparison of values measured for TB-negative and TB-positive patient samples.

Based on our own results, we suggest the following **CUT-OFF levels (NV = Normalised OD value):**

- ⇒ **NV less than 0.8:** **NEGATIVE**
- ⇒ **NV more than 1.2:** **POSITIVE**
- ⇒ **NV in-between 0.8 - 1.2:** **BORDERLINE**

QUALITY CONTROL

The **TUB IgM ELISA** contains standard solutions as an internal control.

The colour of the liquid in the wells containing the standard solutions turn into blue after incubation of the substrate solution (20 minutes after addition of the substrate solution). After addition of the stop-solution the colour changes from blue to yellow. This colour development is considered an internal positive control. It confirms the correct procedural technique.

If the colour of the standard solutions do not turn into blue after incubation of the substrate solution, the test result is invalid.

A clear background in the wells containing the sample diluent is an internal negative control (blank). If a background colour (blue dye) appears in the blank, the test result is invalid.

PERFORMANCE CHARACTERISTICS

Precision:

The intra-assay coefficient of variation (CV) of Human Tuberculosis Test kits (TUB ELISA) was determined by ten-fold repeated measurements of several positive samples. The intra-assay CV is less than 10%.

Diagnostic sensitivity and specificity for serum/plasma:

To determine the diagnostic sensitivity and specificity 695 serum and plasma samples from different regions were evaluated with the **TUB IgM ELISA**. The results of the **TUB IgM ELISA** were compared with clinical outcomes.

As gold standard for the diagnosis of "TB", a pathogen detection based on cultivation was defined (positive control group, a total number of 410 samples). As "negative control", samples of clinically healthy donors and patients with other diseases were measured (285 samples).

Extensive studies on samples of TB patients (culture positive) showed a sensitivity of 36.58% and a specificity of 95.79%.

The positive predictive value (PPV) was 0.9259 with a negative predictive value (NPV) of 0.5122.

LIMITATIONS

Follow the instructions of the test procedure and the interpretation of results carefully!

The **TUB IgM ELISA** is an *in-vitro* diagnostic microtiter plate based Enzyme assay for the quantitative detection of human IgM antibodies to *Mycobacterium tuberculosis* in serum or plasma within 100 minutes.

It is intended for professional *in-vitro* diagnostic use only! For the measurement of other body fluids, this test has not been validated and results may be incorrect.

The IgM antibodies are only formed in the very early stages of the disease by the immune system. Therefore, specific IgM antibodies are detectable only in a very small percentage of all samples from TB patients! The **TUB IgM ELISA** was developed exclusively for the detection of early stages of this disease. The **TUB IgM ELISA** supplements the TUB IgG ELISA and TUB IgA ELISA.

For complete diagnostics, specimens should also be tested for the presence of the antibody classes IgG and IgA. We recommend **measuring the same sample with all TUB ELISA kits** designed for detecting IgG- (REF: LIO-TUB01), IgM- (REF: LIO-TUB02), and IgA class antibodies (REF: LIO-TUB03) in order to increase the sensitivity.

The test is specific for **active tuberculosis (TB)**. The test is not suitable for the detection of so-called latent TB (LTBI) or for contact screening of populations. Infections with other pathogenic mycobacteria cannot be detected with this test.

A definitive clinical diagnosis should be based on an evaluation of all clinical and laboratory findings by a doctor, and not only by the results of one single test. If a patient sample was tested as positive, more confirmatory tests should be performed.

POSITIVE test result needs further confirmation! For final diagnosis take all available clinical results of given patient into consideration (X-ray, microscopy, culture results, serology, clinical signs, PCR, clinical symptoms, IGRA-cytokine detection).

Likewise, a negative test result does not exclude a possible TB infection or disease.

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ATTENTION: The **rheumatoid factors** are antibodies of classes IgG or IgM. In human blood, they can account for up to 5% of all immunoglobulins. Rheumatoid factors, like IgM-rf, binds to IgG antibodies and reacts with immune complexes which are then detected as an IgM molecule. If a patient sample contains rheumatoid factors (IgM-rf), false positive results can occur in pathogen-specific IgM ELISA tests. Therefore, **we recommend to remove all rheumatoid factors** (including IgG antibodies) in the patient sample by treating with a precipitating IgG RF reagent (in kit not included) **prior to performing the TUB IgM ELISA.**

ATTENTION: The OD values for standard solutions must be within the acceptable ranges defined in the kit inserts ($\pm 10\%$, valid range of COA - "Certificate of Analysis").

NOT VALID: If, after 20 minute incubation with the substrate solution, the colour of the liquid in the wells containing the standard solutions does not turn into blue, the test result is invalid.

If, after 20 minute incubation with the substrate solution, a background colour (blue dye) appears in the wells containing the sample diluent (blank), the test result is invalid.

Insufficient sample preparation or sample volume or incorrect handling of the test (wrong sequence of steps during test performance) are the most likely reasons for invalid results. Check again the instructions of sample preparation / test procedure and repeat the test with new microtiter strips. If the problem persists, contact the manufacturer or your local distributor.

Interfering substances:

An excessive amount of lipids in a sample may cause physicochemical interferences due to inhomogeneity. In addition, high concentration of lipids can potentially change the binding behavior of antibodies and thus falsify the results. Therefore, lipaemic samples may not be used.

Haemolysed samples should not be used because certain components of red blood cells pass into plasma or serum and might have potential effects onto the test results.

Recent or ongoing treatment for TB may lead to faulty results. Antibody levels in the blood may diminish rapidly after treatment with anti-TB antibiotics and are maybe too low to detect by this test - even if an infection or disease is present.

If a patient sample contains rheumatoid factors (IgM-rf), false positive results can occur. Therefore, **we recommend to remove all rheumatoid factors** (including IgG antibodies) in the patient sample by treating with a precipitating IgG RF reagent (in kit not included) prior to performing the **TUB IgM ELISA.**

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REF: LIO-TUB03

Rev. 6.0 / 190625

GUIDANCE OF TEST PROCEDURE



ATTENTION! Please follow the instructions for use carefully!

ATTENTION! Avoid contact of skin, eye and mucosa to the stop-solution and TMB-Substrate. The stop-solution is corrosive! The TMB-substrate may damage fertility or the unborn child! Wear safety goggles, gloves and protective clothing!

PREPARATION OF REAGENTS

Before use bring all reagents to room temperature (preferably 15 - 30°C)!



WP Wash buffer (green lid):

Dilute 1:10 with de-ionized or distilled water (1 + 9 volume). If crystals precipitate during the cold storage, the 10 x concentrate solution should be warmed up at 37°C for 15 minutes before dilution.



Sample preparation:

Store the serum or plasma samples at 2 - 8°C for up to 2 days (48 hours). For longer storage, the samples can be stored at temperatures below -20°C. Frozen samples must be thawed prior to testing and well mixed. Avoid repeated freezing and thawing of samples!

For the performance of the test, the samples have to be diluted **1:200** with **PV** sample diluent (white lid)! The standard solutions are ready-to-use and **MUST NOT** be diluted!

TEST PROCEDURE

STEP A:



Pipet 100 µL of 1:200 diluted samples, **PV** sample diluent (blank) and each **STA** standard solution (3 x 2 mL vials with green, yellow and red lid) into the wells (all in duplicates).

INCUBATION: 45 minutes at 37°C.

STEP B:



Washing procedure: Empty the wells of the plate (dump or aspirate) and wash 3 x 300 µL per well with 1:10 diluted **WB** wash buffer. Pipet 100 µL of **CON** conjugate solution (blue lid) into all wells.

INCUBATION: 30 minutes at 37°C.

STEP C:



Danger!

Repeat washing procedure: Wash 3 x 300 µL per well with 1:10 diluted **WB** wash buffer. Pipet 100 µL of **TMB** substrate solution (black lid) into all wells.

INCUBATION: 20 minutes at 37°C (in the dark).

STEP D:



Danger!

Terminate the substrate reaction: Pipet rapidly 100 µL of the **STO** stop-solution (yellow lid) into all wells.

Measure the absorption at 450 nm (optional reference wavelength: 620 nm). The colour is stable for at least 60 minutes.

CUT-OFF levels suggested for TUB IgM ELISA (NV = Normalised OD value):

⇒ **NV less than 0.8:**

NEGATIVE

⇒ **NV more than 1.2:**

POSITIVE

⇒ **NV in-between 0.8 - 1.2:**

BORDERLINE

	Please consult instructions for use		In Vitro Diagnostic Medical Device		Consumables: use by ... (expiry date)		Protect from moisture
	Manufacturer		Do not reuse		Do not use if damaged		Compliant with IVD Directive 98/79/EG
	Store at 2 - 30°C		Batch code		Contains sufficient for <n> tests		Catalogue number