Cat. No.: IR-0001E

Vol. 1.00

M. tuberculosis Antigen ELISA

For the detection and quantitative determination of specific antigen of *M. tuberculosis* secreted in culture broth, human blood or pleural effusion.

96 Tests

QUICK INSTRUCTION:

Preparation:

1. Bring all reagents and samples to room temperature (21-25°C).
2. Prepare 1x Washing Solution by diluting 20x concentrated Washing Solution with reagent grade water.
3. Thoroughly mix all the reagents in the kit and samples.

Test Procedure:

Set enough strips on the strip holder

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Apply **100 µl** of PCs, NC and samples to wells — **100 µl**

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Incubate at **37°C** for **60 min** — **60 min**

↓

Aspirate all liquid from wells. Wash wells for 3 times by 280-300 µl Washing Solution — **WASH**

↓

Add **100 µl** of Conjugate to wells — **100 µl**

↓

Incubate at **37°C** for **30 min** — **30 min**

↓

Aspirate all liquid from wells. Wash wells for 3 times by 280-300 µl Washing Solution — **WASH**

↓

Add **100 µl** of Substrate (TMB) to wells — **100 µl**

↓

Incubate at **37°C** for **10 min** — **10 min**

↓

Add **100 µl** of Stop Solution (1N HCl) to wells — **100 µl**

↓

Read assay plate at 450 nm using 650 nm as reference — **$OD_{450/650}$**

MeDiPro *M. tuberculosis* Antigen ELISA

INTENDED USE:

The **MeDiPro *M. tuberculosis* Antigen ELISA,** is an enzyme linked immunsorbent assay intended for the detection and quantitative determination of specific antigen of *M. tuberculosis* secreted in culture broth, human blood or pleural effusion. This kit may also be used for monitoring the efficiency of tuberculosis antibiotics therapy.

INTRODUCTION:

Tuberculosis (TB) is caused by repeated exposure to airborne droplets contaminated with a rod-shape bacterium, *Mycobacterium tuberculosis*. More than 8 million new cases of Tuberculosis have been diagnosed each year and are responsible for more than three million deaths per year. Almost two and three quarter billion people (2.75 billion) or 33% of population are latently infected with TB.

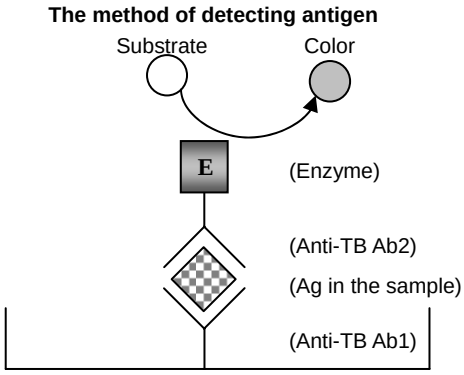
At present, the clinical check (X-ray) coupling with the microscope examination and specimen bacterial culture is the major diagnostic method for the tuberculosis. However, this often takes 4-6 weeks, and the results sometime inaccurate. Among screening infectious diseases, a rapid and accurate diagnostic method of tuberculosis is very important for human health maintain and the disease control.

The **MeDiPro *M. tuberculosis* Antigen ELISA** is developed by Formosa Biomedical Co. and shows superior performance for detecting specific secreted antigens of *M. tuberculosis* from human body fluids.

PRINCIPLES OF THE ASSAY:

1. **Capture of specific antigen in the sample:**

   Individual with activated *M. tuberculosis* infection produces secreted antigens in body fluids. Our ELISA is to quantify the antigens in the any sample containing the specific TB antigens. This is accomplished by incubating sample in a microtiter plate coated with primary anti-TB antibody. The antigens will bind specifically to the antibody on the microtiter plate.



2. **Detection of bound antigen:**

   After incubation for the specified time at the specified temperature, unbound antigens are removed by aspiration and washing. The presence of bound antigen is then disclosed by using an anti-TB antibody 2 conjugated with of horse-radish peroxidase (HRP) and the colorimetric reagent, TMB. The colorimetric result can be determined by the microplate reader. The positivity and the concentration of antigen of the unknown samples are then calculated through an equation and a standard curve.

KIT CONTENTS:

1. ELISA Plate：One strip holder containing 8 wells x 12 strips coated with anti-TB antibody 1.
2. Conjugate：one bottle containing 12 ml of HRP conjugated-anti-TB antibody 2.
3. Washing Solution；20×：one bottle, 50 ml.

4. Positive Control A；20 ng/ml：one vial, 800 µl.
5. Positive Control B；10 ng/ml：one vial, 800 µl.
6. Positive Control C；5 ng/ml：one vial, 800 µl.
7. Positive Control D；1 ng/ml：one vial, 800 µl.
8. Negative Control：one vial, 800 µl.
9. Substrate (TMB): one bottle, 12 ml.
10. Stop Solution (1N HCl): one bottle, 12 ml.
11. Adhesive plate sealing film: one sheet.
12. Instruction manual.

MATERIALS REQUIRED BUT NOT PROVIDED:

1. **Micropipettes and tips** capable of accurately delivering from 25 µl to 1000 µl volumes.
2. **Multi-channel pipettes and tips** capable of accurately delivering from 25 µl to 200 µl volumes.
3. **37°C water bath or incubator.** The temperature must be within 37±2°C.
4. **Microplate reader.** The developed color should be read on an ELISA plate reader equipped with a 450 nm filter and a 650 nm reference filter.

PRECAUTIONS:

1. Safety considerations

1) Please refer to the manufacturer‘s safety data sheet and the product labeling for information on potentially hazardous components.
2) Human source material please handle assay specimens, positive and negative controls as if they are capable of transmitting an infectious agent: Each donor unit used in the preparation of the controls was tested by approved methods for the presence of antibody to human immunodeficiency virus (HIV), hepatitis C virus (HCV) as well as hepatitis B surface antigen (HBsAg) and found to be negative. Because no test method can offer complete assurance that HIV, HCV, HBV or other infectious agents are absent, these materials should be handled with good laboratory practice to avoid skin contact or ingestion.
3) Do not pipette by mouth. Avoid contact with skin and mucous membranes. Avoid splashing and generating aerosols.
4) Do not eat, drink, or smoke in areas in which specimens or kit reagents are handled.
5) Wear disposable gloves throughout the test procedure. Dispose of gloves in the biohazard waste. Wash hands thoroughly afterward.
6) Wipe spills promptly with 1% sodium hypochlorite solution (1 to 5 dilution of liquid household bleach). **Caution:** Liquid waste at acid pH must be neutralized prior to adding sodium hypochlorite solutions (bleach) to avoid formation of poison gas. Contaminated materials should be disposed of in the biohazard waste.
7) Dispose of all specimens and materials used in the **MeDiPro *M. tuberculosis* Antigen ELISA** procedure in the biohazard waste. The recommended method of disposal is to disinfect by autoclaving for 1 hour at 121°C followed by incineration. Mix liquid wastes with an equal volume of 5% sodium hypochloride (liquid household bleach) and let stand for 60 minutes before disposal.
8) The Controls and 20x concentrated Washing Solution contain 0.05% Thimerosal which can be absorbed

through the skin and is a sensitizing agent, please handle carefully.

9) The TMB Substrate Solution contains tetramethylbenzidine, hydrogen peroxide and dimethylsulfoxide, it should be disposed appropriately.

10) The Stop Solution contains hydrochloric acid. Wear disposable gloves and protective glasses when using and disposing of this reagent.

2. Performance considerations

1) Do not use kit components beyond the expired date. **Do not mix components from different lot numbers** except Substrate (TMB) solution, Stop Solution (1N HCl) and 20x Washing Solution. Do not mix with components from other manufacturers.

2) Avoid microbial contamination of reagents. Microbial contamination may interfere with the sensitivity of the assay. When not in use, return all reagents and kit components to refrigerated storage (2 to 8° C).

3) Avoid cross-contamination of reagents. Wash hands before and after handling reagents. Cross-contamination of reagents and/or samples could cause false results. Do not interchange vial or bottle caps and stoppers; this will lead to cross contamination of the reagents. **Do not pour reagents back into vials as reagent contamination may occur.**

4) Shield Substrate (TMB) solution from light. Aliquot only the volume of reagents that is needed. Please do not use Substrate (TMB) when blue color occurred. Do not return used Substrate to the bottles.

5) To avoid substances which may interfere with the assay, use reagent grade quality water (deionized water that is bacteria free) to dilute the 20x concentrated Washing Solution.

STORAGE INSTRUCTIONS:

1. Store **MeDiPro *M. tuberculosis* Antigen ELISA** kits and/or sealed individual reagents at 2 to 8°C.
2. Opened, unused microplate strips must be stored at 2 to 8°C in their original bag with the desiccant provided.
3. Store diluted 1x Wash Solution at room temperature (21 to 25°C) for up to 2 weeks.
4. Avoid storing reagents and specimens in auto-defrost refrigerators.

PREPARATION OF REAGENTS:

1. All the reagents and samples should be brought to **room temperature (21-25°C)** and mix thoroughly before assay. Do not use reagents beyond the stated expiration date marked on the package label.
2. Washing Solution: dilute 1 volume of 20x concentrated Washing Solution with 19 volume of reagent grade water. If the kit is used for multiple times, please prepare appropriate volume for each time.
3. **All samples should be vortexed before use.**

PROCEDURE:

1. Take enough strips and set on strip holder.
2. Add 100 µl of Positive Controls (A, B, C, D), Negative Control and samples to the individual well. Duplication is recommended for each control and blank.
3. Cover the plate with adhesive plate sealing film. Incubate at **37°C** for **60 min**.
4. Aspirate or shake out liquid from all wells. Add 280-300 µl of diluted 1x Washing Solution. Aspirate or shake out and turn plate upside down and blot on paper toweling to remove all liquid. Repeat the wash procedure 2 times

(for a total of 3 washes). Repeat the wash procedure 4 times (for a total of 5 washes) on automated equipment.

****IMPORTANT NOTE: Regarding steps 4 and 7 - Insufficient or excessive washing will result in assay variation and will affect validity of results. Therefore, for best results the use of semi-automated or automated equipment set to deliver a volume to completely fill each well (280-300 µl) is recommended. A total of 5 washes may be necessary with automated equipment. Complete removal of the washing solution after the last wash is critical for the accurate performance of the test. Also, visually inspect to ensure that no bubbles are remaining in the wells.**

5. Add 100 µl of Conjugate to each well.
6. Cover the plate with adhesive plate sealing film. Incubate at **37°C** for **30 min**.
7. Aspirate or shake out liquid from all wells. Wash with diluted 1x Washing Solution 3 times. (5 times for semi-automated or automated equipment)
8. Add 100 µl of Substrate (TMB) to each well.
9. Cover the plate with adhesive plate sealing film. Incubate at **37°C** for **10 min**.
10. Add 100 µl of Stop Solution (1N HCl) to each well to stop the reaction. Tap the plate gently along the outsides to mix contents of the wells thoroughly.
11. Determine absorbance by reading assay plate at 450 nm using 650 nm as reference. The plate may be held up to 30 min after addition of the Stop Solution before reading.

INTERNAL QUALITY CONTROL:

Results of an assay run are valid if the following criteria for the Controls are met:

Average $OD_{450nm-650nm}$ value of control ：

Positive Control A: > 1.50

Positive Control D: > 0.15

Negative Control: < 0.1

If any value is out of the range of above criteria, the assay need repeat.

RESULTS:

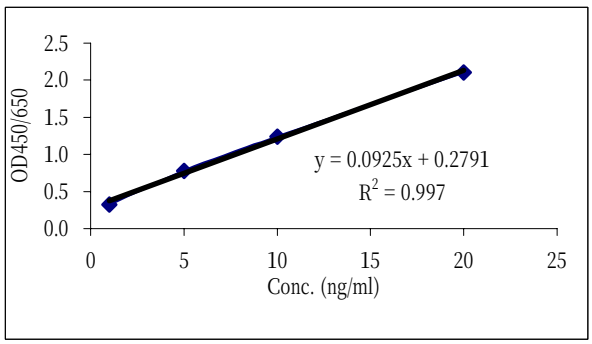
1. Calculate the concentration :

Draw a standard curve on computer analysis software (Microsoft Excel, Lotus123, etc.) by using $OD_{450nm-650nm}$ of positive controls (A, B, C,D) and corresponding concentration (*20, 10, 5, 1 ng/ml*). The concentration of each positive sample can be derived from the equation:

$$Y\ (OD_{450nm-650nm}) = a\ (X) + b$$

For example:

	ng/ml	$OD_{450nm-650nm}$
Positive Control A	20	2.105
Positive Control B	10	1.241
Positive Control C	5	0.775
Positive Control D	1	0.326



2. Cut-off value (C.O.V.) :

   C.O.V. = *Calculated* OD value of 1 ng/ml

   For example:

   Calculated OD value of 1 ng/ml = 0.372

	OD Value
C.O.V.-10%	0.335
C.O.V.(1 ng/ml)	0.372
C.O.V.+10%	0.409

The concentration (ng/ml) can be calculated by the equation of standard curve. If the $OD_{450nm-650nm}$ value of sample is higher than 0.409, the result of this sample is positive, for confirmation of Tuberculosis, further examination on clinical symptoms is necessary; while the $OD_{450nm-650nm}$ value is lower than 0.335, the sample is negative. If $OD_{450nm-650nm}$ value of sample is between 0.335~0.409 (C.O.V.±10%), repeat testing is recommended.

3. Interpretation of positive result :

   There are some implications if positive result is obtained by MeDiPro *M. tuberculosis* Antigen ELISA：

   1) TB antigen carrier without typical symptoms and no infectious ability (non- pleural tuberculosis)
   2) TB antigen carrier without typical symptoms but with infectious ability (pleural tuberculosis)
   3) TB antigen carrier with typical symptoms but without infectious ability (non-pleural tuberculosis)
   4) TB antigen carrier with both typical symptoms and infectious ability (pleural tuberculosis)
   5) Not TB carrier, but the kit is cross-reacted with other similar antigens.

LIMITATIONS OF USE:

1. **MeDiPro *M. tuberculosis* Antigen ELISA** is **not** used for individual who has taken antibiotics for the TB therapy.
2. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results. In particular, correct sample and reagent pipetting, along with careful washing and timing of the incubation steps are essential for accurate results.
3. The results of ELISA immunoassays performed on sample from immunosupressed patients must be interpreted with caution.
4. Samples that remain equivocal after repeat testing should be retested by an alternate method. If results remain equivocal upon further testing, an additional sample should be taken.
5. Results of this test should be interpreted by the physician in the light of other clinical findings and diagnostic procedures.
6. Icteric, lipemic, hemolyzed, or heat inactivated sera may cause erroneous results and should be avoided.
7. Kit procedures or practices outside those in this package insert may yield questionable results.
8. The performance characteristics have not been established for matrices other than culture broth, human serum and pleural effusion.

PERFORMANCE CHARACTERISTICS:

Analytical Specificity (Cross-Reactivity)：To determine the analytical specificity of the **MeDiPro *M. tuberculosis* Antigen ELISA**, 18 mycobacterial species' culture broths were tested. The result showed all strains of BCG and in environmental isolates with the exception of *M. kansasii* and *M. marinum* are obtained negative results.

Species	Results of MeDiPro *TB* Antigen ELISA
TB complex	
M. tuberculosis	+
M. africanum	+
M. bovis	+
BCG sub-strains	
danish	−
gothenburg	−
moreau	−
pasteur	−
tokyo	−
Atypical Strains	
M. abcessus	−
M. avium	−
M. celatum	−
M. chelonae	−
M. intracellulare	−
M. kansasii	+
M. malmoense	−
M. marinum	+
M. smegmatis	−
M. xenopi	−

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