IVD

IGG ANTIBODY TO *MYCOBACTERIUM TUBERCULOSIS*

Catalog No. E050102

96 tests

INTENDED USE

This is an ELISA assay intended for the qualitative detection of biologically active IgG antibodies to *Mycobacteri**um** tuberculosis* in human serum or plasma specimens, acting as an important aid in the diagnosis of *Mycobacterium tuberculosis*.

INTRODUCTION

Mycobacterium tuberculosis (MTB) is a pathogenic bacterial species in the genus *Mycobacterium* and the pathogen of most cases of tuberculosis (TB)[1].

Globally, the TB disease is an enormous problem. TB is the world's most significant infectious disease from a single infection agent[2].

Hence, the early screening and detection of MTB infection is of great benefit to the control of this disease.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

This assay is based on indirect ELISA method. Microtiter wells are pre-coated with recombinant MTB antigens. When test specimens are added, IgG antibodies to MTB, if present in the specimen, bind to the wells. Excessive specimens are washed away prior to the addition of horseradish peroxidase (HRP) labeled anti-human IgG. Afterwards, excessive material is again washed away. Upon addition of the chromogenic substrates, a color will develop only in wells attached with HRP, indicating the presence of IgG antibodies to MTB in corresponding specimens. The enzyme reaction is then stopped with a stop solution. In the final step of the procedure, the absorbance is measured at 450 nm.

MATERIALS PROVIDED

1. Antigen Coated Microtiter Plate: microplate with recombinant MTB antigens coated wells (1 plate, 96 wells)
2. Enzyme Conjugate Reagent: HRP labeled anti human IgG antibodies in stabilizing buffer (1 vial, 11 mL)
3. Negative Control: bovine serum diluted in buffer solution (1 vial, 1 mL)
4. Positive Control: inactivated human serum positive for TMB diluted in buffer solution (1 vial, 0.8 mL)
5. Substrate Solution: hydrogen peroxide (1 vial, 6.2 mL)
6. Chromogen Solution: tetramethylbenzidine (TMB) (1 vial, 6.2 mL)
7. Stop Solution: 1.0 M H_2SO_4 (1 vial, 6.2 mL)
8. Sample Diluent: (1 vial, 11 mL)
9. Wash Fluid Concentrate: PBS-Tween (1 vial, 50 mL, 20×)

MATERIALS REQUIRED BUT NOT PROVIDED

1. Deionised water

2. Vortex mixer
3. Micropipettes and multichannel micropipettes of appropriate volume (the use of accurate pipettes with disposable plastic tips is recommended)
4. Incubator
5. Instrumentation
   a) Automated microplate strip washer
   b) Microplate reader
   or
   c) Fully automated microplate processor
6. Disposable reagent troughs

STORAGE OF TEST KIT AND INSTRUMENTATION

1. Unopened test kits should be stored at 2 – 8℃ upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit (1 year from the date of manufacture). Refer to the package label for the expiration date.
2. Opened components will remain stable for at least 2 months or the labeled expiration date, whichever is earlier, provided they are sealed and stored under conditions as prescribed above.

SPECIMEN COLLECTION, PREPARATION, TRANSPORT AND STORAGE

1. Serum or plasma specimens may be used.
2. Specimens must be collected using correct venipuncture techniques. Sodium azide is not allowed to be added into the specimen as a preservative.
3. Ensure the collected specimens are clotted naturally. Serum specimens must be fully clotted. Any particulate matter must be removed by centrifugation.
4. It must be confirmed that the specimen is not decayed prior to use.
5. Specimens can be stored at room temperature for no more than 8 hours. In case the specimen is not going to be assayed within 8 hours, store at 2 – 8℃. Specimens going to be stored or transported for more than 48 hours must be stored frozen (- 20℃ or lower). Avoid multiple freeze-thaw cycles. After thawing, ensure specimens are thoroughly mixed and brought to room temperature before being assayed.

PRECAUTIONS AND WARNINGS

1. For *in vitro* diagnostic use only.
2. This package insert must be fully understood prior to operation. The operation must be stringently in accordance with the instruction for use.
3. The assay must be conducted away from bad ambient conditions. e.g. ambient air containing high concentration corrosive gas such as 84 disinfectant, sodium hypochlorite, acid, alkaline, acetaldehyde and so on, or containing dust.
4. Unused microplate strips must be stored sealed in a airtight bag, then placed in the original wrap, in order to avoid being dampened.
5. Micropipette tips are not interchangeable to eliminate cross contamination.
6. Specimens added must be mixed thoroughly. The presence of bubbles must be eliminated.

7. The microtiter plate must be washed completely. Each well must be fully injectwash fluid. The strength of injection, however, is not supposed to be too intense to avoid overflow. In each wash cycle, liquids in each well must be dried. The microtiter plate should be stroked onto absorbent paper to remove residual water droplets. It is recommended to wash the microtiter plate with an automated microplate strip washer.
8. Wear disposable gloves when dealing with specimens and reagents. Wash hands after operations. All specimens must be regarded as potentially infectious materials. Waster material must be disposed of safely according to relevant local and national requirements.
9. Reagents must be used within the expiration date. Unused reagents must be sealed immediately, then be stored at 2 – 8℃.
10. Components with different lot numbers are not allowed to be exchanged.

REAGENT PREPARATION

1. Obtain the assays from the fridges. Place at room temperature (18 – 25℃) and equilibrate for at 30 minutes.
2. Carry out a 1:20 dilution of the wash fluid concentrate.
3. Calibrate the temperature of the incubator at 37℃. Only use after the temperature is stabilized.

ASSAY PROCEDURE

1. Obtain a certain quantity of microplate strips, number each well. Leave 1 blank well; add 100 L of negative control to the next 3 wells, then 100 L of positive control to the following 2 wells. Add 100 L of Sample Diluent into each of the rest of the wells, and then add 10 L of specimen into each well with added Sample Diluent.
2. Mix thoroughly by shaking on a vortex mixer for 30 seconds.
3. Incubate at 37℃ for 30 minutes.
4. Wash 6 times (an automated microplate strip washer is recommended), strike the microtiter plate onto absorbent paper at the end of the last wash cycle.
5. Add 100 L of Enzyme Conjugate Reagent into each well except for the blank well.
6. Repeat steps 3 and 4.
7. Add 50 L of Substrate Solution, then 50 L of Chromogen Solution into each well. Mix thoroughly by gently vortexing. Leave the mixture for reaction at 37℃ for 10 minutes without exposure to sunlight.
8. Add 50 L of Stop Solution into each well, mix thoroughly by vortexing, read the results immediately.
9. Immediately after mixing, read the absorbance of each well at 450 nm with a microplate reader using 620 – 630 nm as the reference wavelength. Alternatively, the actual absorbance can be obtained by subtracting the absorbance of each well at 450 nm with the absorbance of the blank well at 450 nm.

CALCULATION OF RESULTS

Test is valid only if absorbance of Positive Control ＞0.7, absorbance of Negative Control ＜ 0.1. absorbance of Positive Control - absorbance of Negative Control ＞0.6. otherwise, the assay should be performed another time. Assay values must be read from the microplate

strip reader.

1. Calculation of the cut-off value

Cut-off value = 0.1 + mean absorbance of Negative Control replicates (in case the mean absorbance of Negative Control replicates ＜0.05, use 0.05 instead of the actual mean）

2. The specimen is positive when the absorbance ≥ the cut-off value, otherwise, the specimen is negative.

INTERPRETATION OF RESULTS

1. When the absorbance is close to the cut-off value, perform another assay and have a dynamic inspection.
2. Assay values from this product cannot be compared directly with assay values from other manufacturers or assays with different methodology.

PERFORMANCE CHARACTERISTICS

1. Sensitivity

The sensitivity is 81.44% (215/264)

2. Specificity

The specificity is 95.94 (591/616)

3. Precision

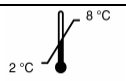
The intra-assay precision ≤15% (n = 10)

LIMITATIONS

1. This assay is only intended as an aid in clinical diagnosis. Assay values must be considered in combination with clinical examination, patient history and other assay values.
2. Specimens containing heterophilic antibodies may interfere with this assay. This type of specimen is not suitable for this assay.
3. Grossly hemolytic or microbial contaminated specimens might generate incorrect results.
4. Heat processed specimens, specimens which have experienced multiple freeze-thaw cycles and other specimens containing precipitations need to be centrifuged for 5 – 10 minutes to remove precipitations. It is recommended to assay freshly made serum or plasma specimens.

SYMBOLS

LOT	**BATCH CODE**
	**USE BY**
	**MANUFACTURER**

	**CONTAINS SUFFICIENT FOR <n> TESTS**
	***IN VITRO* DIAGNOSTIC MEDICAL DEVICE**
	**TEMPERATURE LIMITATION**
	**CATALOGUE NUMBER**
	**CONSULT INSTRUCTIONS FOR USE**

REFERENCES

1. Kenneth James Ryan, C. George Ray, John C. Sherris *Sherris Medical Microbiology : An Introduction to Infectious Diseases;* McGraw-Hill Professional: 2003;
2. Barry R. Bloom *Tuberculosis: pathogenesis, protection, and control;* ASM Press: 1994;

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