Mycobacterium Tuberculosis DNA Detection Kit (Fluorescence PCR) Instructions for Use (V1.0)

[REF] HWTS-RT107A, HWTS-RT107B

[Product Name]

Mycobacterium Tuberculosis DNA Detection Kit（Fluorescence PCR）

[Packaging Size] 20 tests/kit, 50 tests/kit

[Research Use Only]

It is suitable for qualitative detection of Mycobacterium tuberculosis DNA in human sputum samples.

Mycobacterium culosis, referred to as Tubercle bacillus(TB), is a class of obligate aerobic bacteria with positive acid-fast staining. It is amastigote and has fimbriae and microcapsules but not forms spores. Its bacterial wall has neither teichoic acid of gram-positive bacteria, nor lipopolysaccharide of gram-negative bacteria. Mycobacterium tuberculosis, which is pathogenic to humans, is generally considered to be human, bovine, and African. Its pathogenicity may be related to the inflammation caused by the proliferation of bacteria in tissue cells, the toxicity of bacterial components and metabolites, and the immune damage to the bacterial components. Pathogenic substances are associated with capsules, lipids and proteins. Mycobacterium tuberculosis can invade susceptible organisms through the respiratory tract, digestive tract or skin injury, causing tuberculosis of various tissues and organs, of which the most common is pulmonary tuberculosis through the respiratory tract. It usually occurs in children, and presents with symptoms such as low-grade fever, night sweats, and a small amount of hemoptysis. Secondary infection is mainly manifested as low-grade fever, night sweats, and hemoptysis. Mostly it is long-term chronic disease, and a few are acute. Tuberculosis is one of the top ten causes of human death worldwide. In 2018, about 10 million people around the world were infected with Mycobacterium tuberculosis, of which about 1.6 million died.

IS6110 is an inserted nucleotide sequence discovered by Thierry et al. in 1990 in Mycobacterium tuberculosis. IS6110 is present in multiple copies of a sequence in the Mycobacterium tuberculosis, Mycobacterium africanum, Mycobacterium bovis, and BCG genes. IS6110 is relatively stable in the strains in which it exists, and will not change the IS6110 DNA fingerprints when the strains develop drug-resistant mutations. The 38KD protein antigen is a phosphate transporter and contains 6 epitopes targeted by monoclonal antibodies specific for Mycobacterium tuberculosis. The diagnostic specificity of the reported 38KD is 88%-100%.

[Test Principles]

This kit uses the methods of combination of PCR amplification and fluorescent probe, selects the specific conserved region of Mycobacterium tuberculosis IS6110 and 38KD antigen sequence as the detection target gene region, and designs specific primer and probes for fluorescent detection. The specific probes 5' is labeled FAM (IS6110 and 38KD), VIC (HEX) (internal control gene) fluorophore, and the 3' quencher is BHQ1. In the process of PCR amplification, specific primers and probes bind to their respective target sequences. During the amplification process, the formation of PCR products is achieved through the DNA polymerase activity and 5'-3' exonuclease activity of Taq enzyme. Meanwhile the accumulation of fluorescent signals is completely synchronized. And the qualitative detection of sample nucleic acid is realized through one reaction buffer.

[Main Components]

S/N	Component(20 tests/kit)	Strength	Quantity	Component Description
1	TB Reaction Buffer	400μL/vial	1 vial	Mycobacterium tuberculosis IS6110 and 38KD, internal control specific primers, fluorescent probes, Taq enzyme, UDG enzyme, reaction buffer, etc.
2	TB Positive Control	400μL/vial	1 vial	IS6110, 38KD and internal control mixed plasmid diluent
3	TB Blank Control	400μL/vial	1 vial	DNase/RNase free H_2O

S/N	Component(50 tests/kit)	Strength	Quantity	Component Description
1	TB Reaction Buffer	1mL/vial	1 vial	Mycobacterium tuberculosis IS6110 and 38KD, internal control specific primers, fluorescent probes, Taq enzyme, UDG enzyme, reaction buffer, etc.
2	TB Positive Control	1mL/vial	1 vial	IS6110, 38KD and internal control mixed plasmid diluent
3	TB Blank Control	1mL/vial	1 vial	DNase/RNase free H_2O

Note: Components of different batches cannot be mixed or interchanged.

Reagents need but not provided: Stroke-physiological saline solution, 4% NaOH, Macro & Micro-Test Viral DNA/RNA Kit(HWTS-3001, HWTS-3004-32, HWTS-3004-48) and Macro & Micro-Test Automatic Nucleic Acid Extractor(HWTS-3006), Nucleic Acid Extractor(1000020261) and High Throughput Automated Sample Preparation System(MGISP-960) by MGI, Nucleic Acid Extraction or Purification Reagent(YDP302) by Tiangen Biotech(Beijing) Co.,Ltd.

Consumables need but not provided: RNase/DNase-free Tips, disposable gloves, RNase/DNase free EP tubes, Centrifuge, Magnetic Stand, 8-tube strips for PCR.

[Storage Conditions and Shelf-life]

Storage condition: The kit should be stored below -18℃ protected from light. The shelf life is 12 months. The number of repeated freezing and thawing should not be more than 4 cycles. After opening, it should be stored below -18℃. If it is stored 2~8℃, please use it within 1 week.

Transportation: The kit is stable for 5 days in a shipping box containing dry ice.

See the packaging label for the production date, production batch number and expiration date.

[Applicable Instruments]

Applied Biosystems 7500 Real-Time PCR Systems, Applied Biosystems 7500 Fast Real-Time PCR Systems, QuantStudio®5 Real-Time PCR Systems, SLAN-96P Real-Time PCR Systems(Shanghai Hongshi Medical

Technology Co., Ltd.), LightCycler®480 Real-Time PCR system, LineGene 9600 Plus Real-Time PCR Detection System(FQD-96A, Bioer technology), MA-6000 Real-Time Quantitative Thermal Cycler (Suzhou Molarray Co., Ltd.) , BioRad CFX96 Real-Time PCR System, BioRad CFX Opus 96 Real-Time PCR System.

[Requirements for Samples]

1. Sample Collection

For those with less sputum, let the patient rinse his mouth after waking up in the morning, then take a few deep breaths, cough up the sputum from the lungs, and collect it in a clean container for testing as soon as possible. For those with much sputum, first ask the patient to cough lightly and cough out the old sputum retained in the trachea, and then take a deep breath several times to cough up fresh sputum from the depths of the lungs, and send it for testing immediately. If there is no sputum, you can go to the hospital for atomization inhalation to induce sputum, and then send it for testing.

2. Precautions for sample collection: Avoid contamination during sample collection, storage and transfer.

Sample safety: All samples are considered to be potentially infectious, and operations are performed in accordance with relevant national standards.

3.Storage

The sputum samples to be tested should not be stored at 2−8°C for more than 24 hours; stored at temperatures below −18°C for no more than three months; can store at temperatures below −70°C for long time. Repeated freezing and thawing should be avoided.

[Test Procedure]

Please read this test procedure carefully before use.

1. Reagent Preparation

(1) Take out the TB reaction buffer in the kit, place it at room temperature. After it is completely thawed, shake and mix, centrifuge for later use.

(2)Calculate the number of samples to be prepared, N (N = number of samples + 1 tube of positive control + 1 tube of blank control), dispense 20μL/well TB reaction buffer into the 8-tube strips for PCR. The remaining TB reaction buffer should be immediately stored below -18°C. If it is stored 2~8°C, please use it within one week.

2.Sample Process

2.1 Sputum Sample

Sputum processt: Add 4 times the volume of 4% NaOH to 1-3 mL of sputum, shake well, incubate at 37°C for 30 min to liquefy, and centrifuge at 13,000 rpm for 15 min. Discard the supernatant, add 1 mL of stroke-physiological saline solution to the sediment, shake well, centrifuge at 13,000 rpm for 10 min, discard the supernatant, and repeat again.

Recommended extraction reagent: Nucleic Acid Extraction or Purification Reagent(YDP302) by Tiangen Biotech(Beijing) Co.,Ltd. The extraction should be extracted from step 2 according to the extraction instructions, and the recommended elution volume is 100 μL.

Recommended extraction reagent: Macro & Micro-Test Viral DNA/RNA Kit(HWTS-3001, HWTS-3004-32, HWTS-3004-48)., The extraction should be conducted according to the extraction instructions. The extraction sample volume is 200μL , and the recommended elution volume is 80μL.

Recommended extraction reagents: Nucleic Acid Extractor(1000020261) and High Throughput Automated Sample Preparation System(MGISP-960) by MGI should be extracted according to the extraction instructions. The extraction volume is 160μL, and the recommended elution volume is 60μL.

TB positive control and TB blank control need to be extracted in parallel, and the volume of one extraction is 200 μL.

3.Add the Samples

Add 5 μL each of the blank control, positive control, and DNA of the sample to be tested processed in step 2 respectively to the set reaction tube, press the tube cap tightly, and centrifuge briefly.

4.PCR Amplification

4.1 Instrument Channel and Sample Volume Selection

1) Select FAM channel (Reporter: FAM, Quencher: None) to detect target (IS6110 and 38KD) nucleic acid DNA;

2) Select the VIC (HEX) channel (Reporter: VIC (HEX), Quencher: None) to detect the internal control;

3) Reference Dye: Select None (applicable to ABI series instruments only); for specific detection channel settings, please refer to the instruction manual of each instrument.

4) The Sample Volume is 25 μL.

4.2 PCR Amplification Conditions Setting

Step	Cycles	Temperature	Time	Collect Fluorescent Signals or Not
1	1 cycle	50℃	2 mins	No
2	1 cycle	95℃	10mins	No
3	40 cycles	95℃	15 secs	No
		60℃	40 secs	Yes

5.Result Analysis

5.1 Applied Biosystems 7500 Real-Time PCR Systems, Applied Biosystems 7500 Fast Real-Time PCR Systems Baseline and Threshold Setting Method

Baseline setting: The starting point of the baseline is set to 3 and the end point is set to 15.

Threshold setting: The threshold of each fluorescent channel should be set separately. The blank control should be selected firstly and click off the Automatic standard curve by changing the option from "☑Auto"to "□Auto". Set the threshold manually just above the maximum level of blank control FAM/VIC(HEX) channel curve (random noise curve).

5.2 SLAN-96P Real-Time PCR Systems(Shanghai Hongshi Medical Technology Co., Ltd.) Baseline and Threshold Setting Method

Baseline setting: The starting point of the baseline is set to 6 and the end point is set to 12.

Threshold setting: The threshold of each fluorescent channel should be set separately. In setting the threshold for a channel, change the configuration of baseline optimization in basic parameter from automatic to manual. Then, manually set the threshold just above the maximum level of amplification curve at FAM/VIC(HEX) channel of blank control(random noise curve).

5.3 QuantStudio®5 Real-Time PCR Systems Baseline and Threshold Setting Method

Baseline setting: Baseline is set as default.

Threshold Setting: Thresholds automatically set by the software can be used. It can be adjusted manually. In

setting threshold, click[Show Plot Setting], select the target gene to view and click the “Show: Threshold” .
Adjust the threshold through dragging it by mouse or inputting values directly, then, click [Analyze].

5.4 LightCycler®480 Real-Time PCR System Baseline and Threshold Setting Method

Baseline setting: Baseline is set as default.

Threshold setting: It can be adjusted through slightly improving the standard curve error value by manually moving the threshold line up or down, fitting the line to the exponential portion of the amplification curve, higher than while horizontally paralleling the amplification curve of Blank control. Click [Analysis] to get results and [Report] to present them.

5.5 LineGene 9600 Plus Real-Time PCR Detection System （FQD-96A， Bioer technology) Baseline and Threshold Setting Method

Baseline setting: Baseline is set as default.

Threshold Setting: Thresholds automatically set by the software can be used. When it is necessary to manually set the threshold, manually drag the threshold line to just exceed the highest point of the amplification curve (irregular noise line) of each channel of the normal blank control. The threshold of each channel should be set separately: first select the desired channel, then drag the threshold line.

5.6 MA-6000 Real-Time Quantitative Thermal Cycler (Suzhou Molarray Co., Ltd.) Baseline and Threshold Setting Method

Baseline setting: Baseline is set as default.

Threshold Setting: Thresholds automatically set by the software can be used. When it is necessary to manually set the threshold, the box in front of "Auto Threshold" must be kept unchecked, and then manually drag the threshold line to just exceed the highest point of the amplification curve (irregular noise line) of each channel of the normal blank control. The threshold of each channel should be set separately: first select the channel to be set, and then drag the threshold line.

5.7 BioRad CFX96 Real-Time PCR System, BioRad CFX Opus 96 Real-Time PCR System Baseline and Threshold Setting Method

Baseline setting: Choose the default baseline.

Threshold Setting: Thresholds automatically set by the software can be used. When it is necessary to manually set the threshold, the box in front of "Auto Threshold" must be kept unchecked, and then manually drag the threshold line to just exceed the highest point of the amplification curve (irregular noise line) of each channel of the normal blank control. The threshold of each channel should be set separately: first select the channel to be set, and then drag the threshold line.

6.Quality Control

1) Blank control: No Ct value is detected at FAM channel, and no Ct value or Ct value >35 is detected at VIC (HEX) channel.

2) Positive control: Positive controls have amplification curves in FAM and VIC (HEX) channels and Ct values ≤35.

The above requirements must be satisfied in the same experiment, otherwise this experiment will be invalid.

[Cut-off Value]

The critical value detected by the kit was analyzed by ROC curve analysis and percentile methods. The cut-off value of this kit for detecting mycobacterium tuberculosis is Ct value ≤39, and the cut-off value of internal control is Ct value ≤35.

[Interpretation]

1. If the FAM channel of the test sample has an obvious amplification curve and the Ct value≤39, the test is positive for tuberculosis.
2. If the FAM channel of the test sample has no amplification curve, the VIC (HEX) channel has an obvious amplification curve and the Ct value ≤35, the test is negative for tuberculosis.
3. If the FAM channel of the test sample has an obvious amplification curve, and the Ct value>35 or no Ct value at VIC (HEX) channel, the test is invalid and needs to be re-extracted. If the re-tested Ct value of the VIC/HEX channel(internal control) ≤35, the results should be judged as above 1-2. If the re-tested Ct value of the VIC/HEX channel(internal control) >35 or there is no Ct value. The result is invalid. It is recommended to increase the sample size or use other methods for verification.

[Limitations]

1. The test results of this kit are for research use only.
2. Unreasonable sample collection, transport, storage and processing may lead to false test results.
3. Contamination of amplification products and cross-contamination between samples of nucleic acid extraction are prone to false positive results. Therefore, laboratories should be equipped with equipment and operators in strict accordance with the Specifications and should be carried out in strict accordance with the requirements of the instructions.
4. A negative result does not mean that the patient is not infected by mycobacterium tuberculosis. Negative results may be caused by: ① unreasonable sample collection, transportation and processing, low pathogen titer in the sample; ② mutation of pathogen detection target sequence,;③ other unverified interfering factors such as taking antibacterial, etc;④ the patient is infected by other viruses or bacteria.

[Performance]

1. The appearance of the kit is intact and the component is complete, and the liquid component is clear, transparent and free of insoluble matter. The filling amount should not be less than the labeled amount. The packaging label should be clear and easy to identify.
2. The test results of 15 national negative references are all negative. It has been verified that this kit does not cross-react in the detection of human genome and other non-tuberculous mycobacteria and pneumonia pathogens.
3. The test results of 15 national positive references are all positive.
4. The LoD of this kit is 10^0 bacteria/mL, and the national LoD references S1-S4 can all be detected. S1-S4 are 10^0 bacteria/mL, 10^1 bacterias/mL, 10^2 bacterias/mL, 10^3 bacterias/mL.
5. Detect 10 national repeatability references (CV), CV≤5.0%.

[Precautions]

1. Mycobacterium tuberculosis samples are contagious. The collection, storage, transportation and processing of samples should follow the corresponding national regulations and biosafety regulations. Relevant protective measures must be taken during sample processing to ensure that laboratory personnel Safety.
2. Reaearch use only. Please read the instructions carefully before the experiment, and the relevant laboratory management specifications are strictly implemented in accordance with the management regulations for gene amplification testing laboratories promulgated by the administrative department.

3. The results of this kit will be affected by the source and quality of the sample itself, sample transportation conditions, sample pretreatment and other factors, as well as by the quality of DNA extraction and the limitations of current molecular biology technology, which may lead to false results. Users must be aware of potential errors and limitations of accuracy that may exist in the detection process.

4.All reagents in this kit are specially prepared for the above detection. Random replacement of any reagents in the kit may affect the use. Components of different batches of kits cannot be mixed with each other.

5. Please strictly partition the experimental area:

First area: Pre-PCR preparation area—prepare the reagents required for amplification

Second area: Sample processing area - processing of samples to be tested and references;

Third area: Test area-PCR amplification detection.

Items in each area are dedicated and should not be used interchangeably to avoid contamination; please clean the workbench immediately after the experiment.

6. Sample lysates stored below -18°C or below -70°C should be thawed at room temperature before adding samples, and used after brief centrifugation.

7. The reaction tube containing the reaction buffer should be covered with the cap, put into a sealed bag, and then transferred to the sample processing area. The samples should be completely dropped into the reaction buffer when adding the samples, and no sample should adhere to the tube wall. Close the cap as soon as possible after adding the samples.

8. During the experiment, prevent the contamination of reagents by exogenous DNA, and pay attention to adding blank control first, then adding the DNA of the sample to be tested, and finally performing the operation of the positive control. It is recommended to use special filter tips when preparing reaction buffer and adding DNA template.

9. After the amplification, immediately take out the reaction tube, seal it in a special plastic bag, and discard it at the designated place after sterilization.

10. The centrifuge tubes and tips used in the experiment must be free of RNase and DNase. The workbench and various experimental supplies are regularly disinfected with 1% sodium hypochlorite, 75% alcohol or UV light.

[References]

[1]. Programme W . Global tuberculosis control: WHO report [annual].[J]. Who Global Tuberculosis Programme, 2003.

[2]. Kocagoz T , Yilmaz E , Ozkara S , et al. Detection of Mycobacterium tuberculosis in sputum samples by polymerase chain reaction using a simplified procedure.[J]. Journal of clinical microbiology, 1993, 31(6):1435-1438.

[3]. Mikhailovich V , Lapa S , Gryadunov D , et al. Identification of Rifampin-Resistant Mycobacterium tuberculosis Strains by Hybridization, PCR, and Ligase Detection Reaction on Oligonucleotide Microchips[J]. Journal of Clinical Microbiology, 2001, 39(7):2531-2540.

[4]. Miller M B , Popowitch E B , Backlund M G , et al. Performance of Xpert MTB/RIF RUO Assay and IS6110 Real-Time PCR for Mycobacterium tuberculosis Detection in Clinical Samples[J]. Journal of Clinical Microbiology, 2011, 49(10):3458-62.

[Index of Symbols]

Symbols	Meanings	Symbols	Meanings
RUO	RESERCH USE ONLY	REF	CATALOGUE NUMBER
	MANUFACTURER		CAUTION
	USE BY DATE		TEMPERATURE LIMITATION
LOT	BATCH CODE		CONSULT INSTRUCTIONS FOR USE
	DATE OF MANUFACTURE		KEEP AWAY FROM SUNLIGHT
	KEEP DRY	Σ	CONTAINS SUFFICIENT FOR N TESTS

[Basic Information]

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