Mycobacterium tuberculosis DNA FLUORESCENCE DIAGNOSTIC KIT
(PCR-FLUORESCENCE PROBING)

For Professional Use

IVD

Read the pack Insert before use provided along with the kit

REF-Mtdf-48

INTENDED USE

Mycobacterium tuberculosis (TB) is a pathogenic bacterium that causes tuberculosis. It is likely to infect all human tissues and organs, especially the lungs. Early diagnosis and treatment are important for effective control of TB. In recent years, with the development of Molecular Biology, nucleic acid fluorescence quantitative PCR method based on the mycobacterium tuberculosis nucleic acid has drawn more and more attention from researchers.

This diagnostic kit is an *in vitro* nucleic acid amplification test for the detection of *Mycobacterium tuberculosis* (TB) DNA in human sputum. It is intended for use as an aid in diagnosing a TB infection.

TEST PRINCIPLE

The diagnostic kit uses a nucleic acid lysis buffer to allow rapid lysis and release of TB-DNA from a sputum specimen. By applying real-time fluorescence quantitative PCR technology, this test utilizes a pair of specific primers which are designed to target at a conserved sequence of TB-DNA, a specific fluorescence probe, accompanied with PCR mix, to achieve fast detection of TB-DNA through fluorescent signal changes. The PCR detection system uses UNG enzyme + dUTP contamination-proof system, which can fully degrade possible unwanted side-products, to avoid a false positive result.

PACK SIZE: 48Tests/Kit

COMPONENTS OF THE KIT

S. no.	Reagent name	Specification & quantity	Main ingredients
1	DNA lysis buffer	2.5ml / Tube ×1 Tube	KCl, SDS, Surfactin
2	Enzyme mixture	96µl / Tube ×1 Tube	DNA Polymerase , Uracil DNA *glycolase*
3	TB PCR Mix	912µl/Tube x 2 Tube	Primer Probe, dNTPs,Mg^{2+}, PCR Buffer
4	TB- Quantitative Reference-A	50µl / Tube ×1 Tube	Quantitative TB Positive specimen(Inactivated)
5	TB- Quantitative Reference-B	50µl / Tube ×1 Tube	Quantitative TB Positive specimen(Inactivated)
6	TB- Quantitative Reference-C	50µl / Tube ×1 Tube	Quantitative TB Positive specimen(Inactivated)
7	TB- Quantitative Reference-D	50µl / Tube ×1 Tube	Quantitative TB Positive specimen(Inactivated)
8	TB- Negative Control	50µl / Tube ×1 Tube	Sterile saline
9	TB Positive control	50µl / Tube ×1 Tube	Quantitative TB Positive specimen(Inactivated)
10	TB-Internal Control	50µl / Tube ×1 Tube	Positive internal reference(cloning plasmid without TB target sequence)

NOTE:

(1) Do NOT mix components from different lots.

(2) All biological specimens in the detection kit should be handled as if infectious though they have been inactivated.

(3) Self-prepared reagent: sterile saline and 4% NaOH solution.

STORAGE CONDITION AND TERM OF VALIDITY

The detection kit should be stored in sealed pouch at -20±5^{0}C, protected from light. The term of validity is 12 months. Care should be taken to avoid re-freezing and re-thawing.

APPLICABLE INSTRUMENT

The diagnostic kit is applicable to fluorescence PCR instruments such as ABI7500 and Mx3000P.

SPECIMEN REQUIREMENTS

1. Applicable specimen type: Human Sputum

2. Collection of specimen:

It is recommended to collect the first sputum in the morning. First rinse mouth with water. Make a hard cough and collect the sputum in the deep and keep it in a sterile collection tube. Seal it and send it for detection.

3. Storage and delivery of specimens:

Specimens collected via the above-mentioned method can be used for immediate detection, or stored at 2-8^{0}C for not more than 24 hours, or below -20^{0}C for a longer term of storage. Care should be taken to avoid re-freezing and re-thawing. Specimens should be transported in a sealed frozen pitcher with ice or in a sealed foam box with ice.

TEST METHOD

1. Preparation of reagent (Performed at "reagent preparation region")

1.1 Take out each component from the detection kit and place them at room temperature. When the components' temperature has reached room temperature, mix them for later use.

1.2 Refer to quantities of test specimens, negative controls and positive controls, and quantitative references A-D, pipette appropriate quantities of PCR-mix, enzyme mixture and internal control (PCR mix 38 µl/test+ enzyme mixture 2µ l/test+ internal control 1µ l/test), fully mix them and centrifuge it instantaneously for later use.

2. Processing and adding specimens (performed at "specimen processing region")

2.1 Processing specimen

Add into specimen 4% NaOH solution whose volume equals 2-3 times of specimen. Vortex it and hold it for 30 min. to allow it liquefy. Take 500µl liquefied specimen to a 1.5 ml centrifuge tube (avoid aspirating apparent solid impurities). Centrifuge it at 12000 rpm for 3 minute. Aspirate and discard the supernatant. Add 1 ml sterile saline and centrifuge it at 12000 rpm for 3 minute. Aspirate and discard the supernatant. Add into 50µl nucleic acid lysis buffer. Treat it at 100^{0}C for 10 min. Centrifuge it at 12000 rpm for 3 min. Take the supernatant as specimen for later use.

2.2 Adding specimens (negative controls and specimens are processed in synchronization)

2.2.1 Add 5µl of specimen, Negative control, Positive control and Quantitative references respectively into each PCR reaction tube

2.2.2 Add 40µl of PCR-mix into each tube. Remove the bubbles and cover the tube. Centrifuge it at 2000 rpm for 30 seconds.

3. PCR Amplification (performed at "amplification and analysis region"; refer to user's manual for each instrument to do the settings)

3.1 Place the PCR reaction tube into the specimen well of the amplification device. Set negative control, positive control, quantitative references A-D and unknown specimens in corresponding sequence, and set specimen names.

3.2 Select PCR test channel:

3.2.1 For ABI, Stratagene series:

(1) Select FAM channel (Reporter: FAM, Quencher: None) to test: TB-DNA;

(2)Select HEX or VIC channel (Reporter: HEX/VIC, Quencher: None)to test TB-internal control;

(3) Set passive reference: none;

SET SAMPLE VOLUME: 50.

3.2.2 For Roche Light Cycler 480:

Choose "New Experiment". Click "Dual Color Hydrolysis Probe/ UPL Probe" in the drop-down menu of setup panel. Do the following in the drop-down menu of "Customize":

(1) Select FAM channel to test TB-DNA.

(2) Select VIC/HEX/Yellow 555 channel to test internal control.

(3) Set reaction volume: 50.

Set cycle parameters (the time parameter varies according to instruments):

ABI, STRATAGENE SERIES:

S. no	Step	Temperature	Time	Cycle
1	UNG enzyme reaction	50° C	2 min	1
2	Taq Enzyme activation	94°C	5min	1
3	Denaturation	94°C	15 sec	45
	Annealing, Extension, Fluorescence collection	57°C	30 sec *	
4	Device cooling (optional)	25° C	10 sec	1

NOTE:

Due to the device ABI 7500's technical specification, it can't be set at 30 seconds but 31 seconds. When the setting is completed, save settings, and carry out the reaction procedure.

RESULT ANALYSIS (Refer to user's manual for each instrument to make the setting)

When the reactions are completed, results will be saved automatically. After analysis, adjust Start, End and Threshold values of Baseline of the graph (users can adjust according to the actual situation. Start value can be set between3-15, end value between5-20. Adjust the amplification curve of negative control to be flat or below threshold). Click "Analyze" to implement the analysis and make sure each parameter satisfies the requirements given in "Quality Control". Go to "Plate" window to record the detected Ct value.

QUALITY CONTROL

5.1 TB-Negative Control: no display of Ct value, but the internal control is detected as positive. (Ct value ≤40)

5.2 TB-Positive Control: detected Ct value ≤30

5.3 Four TB Quantitative References: all are tested as positive

5.4 The above requirements must be satisfied at the same time in the same test, otherwise, the test is treated as invalid, and needs to be re-tested.

REFERENCE RANGE

Through the research on reference values, the Ct reference value of target gene is 39, and the Ct reference value of internal control is determined to be 40.

EXPLANATION OF DETECTION RESULT

Determination of Negative and Positive results:

(1) Specimens which are detected with a Ct value ≤39 are reported as TB DNA-positive.

(2) For specimens which are detected with a Ct value >39 and internal control is detected as positive (Ct value ≤ 40), report that TB DNA is lower than detection limit. If the Ct value of internal control > 40 or there is no display of Ct value, the test result is invalid. An investigation should be conducted to find out reasons and retest it. (If retests still show invalid result, please contact **Bhat Bio-Tech India Pvt. Ltd. Bangalore.**)

LIMITS OF DETECTION METHOD

Detection result of specimen is related to specimen collection, processing, delivery and storage quality. Any deviation from the stated procedure will lead to an inaccurate detection result. Cross-contamination during specimen processing may also result in a false-positive result.

PRODUCT PERFORMANCE INDEX

When the kit is used to detect the enterprise's work references, the consistency rate for both negative and positive reaches 100%. Precision test shows excellent reproducibility in both intra-batch and inter-batches with its coefficient of variation of Ct value <10%, and its coefficient of variation of concentration <50%. The sensitivity of this kit is 1 bacterium/ml. It shows no cross-reaction with *Mycobacterium Avium, Land Mycobacterium, Amur Mycobacterium, Mycobacterium Kansasii, Asian Mycobacterium, M.scrofulaceum Mycobacterium, Gordon Mycobacterium, Turtle pus Mycobacterium, Accidental Mycobacterium, Mycobacterium phlei, Brazilian Nocardia, Corynebacterium pekinense, Pneumococcus, Legionella pneumophilia, Bordetella pertussis, MP, EBV and respiratory syncytial virus.*

PRECAUTIONS

(1) The product can only be used for *in vitro* diagnosis. Please read carefully the product manual before operation.

(2) Please familiarize with and learn the operation procedures and precautions for each instrument before performing tests. Conduct quality control for each test.

(3) Laboratory management shall strictly follow management practices of PCR gene amplification laboratory; test staff must receive professional training; testing process must be performed in separated regions; all used consumables should be of sterile single use; specific instruments and devices should be used for each stage. All lab devices used in different stages and regions should not be cross-used.

(4) All specimens for detection should be handled as if infectious. Wear protective disposable gloves, laboratory coats and eye protection when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and test reagents. Handling of waste must meet relevant requirements outlined in "Common Criteria of Bio-safety for Microbiology Biomedical Laboratory" and "Medical Wastes Management Regulations" by Health Department.

(5) Before use, all reagents must be fully thawed at room temperature and mixed thoroughly.

BIBLIOGRAPHIES

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[3] van Doorn HR, An DD, de Jong MD, Lan NT, et al.Fluoroquinolone resistance detection in Mycobacterium tuberculosis with locked nucleic acid probe real-time PCR.Int J Tuberc Lung Dis. 2008(7):736-42.

[4] B.J. Renton, P. D. Morrell.Direct real-time PCR examination for Mycobacterium tuberculosis in respiratory samples can be cost effective.Health,200 9,11:63-66.

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