TB/NTM Nucleic Acid Detection Kit (Lyophilized)

Use Manual

Cat.No.: RH018B

Label	Description	Label	Description
	Contains sufficient for <N> tests		In vitro diagnostic
	Batch number		Consult instruction before use
	Date of manufacturing		European Authorized Representative
	Date by which the device should be used		Temperature limitation
	Name and address of Manufacturer		

【PRODUCT NAME】

TB/NTM nucleic acid detection kit (lyophilized)

【SIZE】

24Tests/Kit, 50Test/kit

【INTENDED USE】

Mycobacterium tuberculosis (TB) and Non-tuberculosis mycobacteria (NTM) are pathogens that seriously endanger human health, mainly through the respiratory tract to cause pulmonary infectious disease--pulmonary tuberculosis (PTB).The people with diabetes, tumors, and people taking immunosuppressive drugs for a long time are prone to develop tuberculosis. NTM has clinical symptoms similar to the clinical manifestations of TB. The common symptoms are fever, which usually lasts for several weeks, cough, dyspnea, chest pain, and chronic hemoptysis or sputum.

The kit is designed to qualitatively detect DNA of TB or NTM in samples from suspected cases of Mycobacterium tuberculosis or Non-tuberculosis mycobacteria infection, and to detect Mycobacterium tuberculosis Non-tuberculosis mycobacteria group and differentiate five most common Non-tuberculosis mycobacteria types: Mycobacterium avium(MAV), Mycobacterium intracellulare(MIN), Mycobacterium abscesses(MAB), Mycobacterium massiliense(MMS), Mycobacterium Kansasii(MKA). This kit can be used for auxiliary diagnosis of diseases caused by Mycobacterium tuberculosis and Non-tuberculous bacteria, and can not be used as a routine in vitro diagnostic (IVD) reagent in clinic.

【PRINCIPLE OF DETECTION】

This product is based on Taqman PCR detection system with fluorescent probes. During the amplification of the sample template, the Taqman probe is degraded due to the 5'-3' polymerase activity and exonuclease activity of Taq DNA polymerase, and then the fluorescent reporter gene and quencher gene are separated, enabling the instrument to detect the fluorescent signal . **In PCR Mix A**, the FAM channel detects Mycobacterium tuberculosis, the ROX channel detects Non-tuberculosis mycobacteria, the CY5 channel detects Mycobacterium Kansasii(MKA), and the HEX channel detects the Internal reference gene. **In PCR Mix B**, the FAM channel detects Mycobacterium avium(MAV), the HEX channel detects Mycobacterium intracellulare(MIN), the ROX channel detects Mycobacterium

abscesses(MAB), and the CY5 channel detects Mycobacterium massiliense(MMS). The internal reference of the kit act as quality control during the whole detection process from the beginning of sample collection to avoid false negative results.

【PRODUCT CONTENTS】

Component	Volume	Package	Ingredients
TB/NTM PCR Mix A (Lyophilized)	15μl×24	3×0.2ml or 0.1ml 8 well-strip tube	Contains primers and fluorescent probes of TB, NTM, MKA and Internal Control, dNTPs, $MgCl_2^+$,Taq DNA polymerase
	750μl	1×bottle	
TB/NTM PCR Mix B (Lyophilized)	15μl×24	3×0.2ml or 0.1ml 8 well-strip tube	Contains primers and fluorescent probes of MAV, MIN, MAB, and MMS, dNTPs, $MgCl_2^+$,Taq DNA polymerase
	750μl	1×bottle	
Dissolving solution	1200μl	1×1.5ml tube	/
Negative Control	200μl	1×1.5ml tube	0.9%NaCl
Positive Control	40μl×2	2×0.2ml tube (Lyophilized)	Plasmid of TB, NTM, MAV, MIN, MAB, MMS and MKA DNA fragment
Internal Control	140μl×2	2×0.2ml tube (Lyophilized)	Exogenous IC template

【STORAGE & SHELF LIFE】

Stored：2~30℃. And the kit is stable for 12 months

【INSTRUMENTS】

Real-time fluorescent PCR instrument, such as ABI7500, Roche LC480\COBAS Z480, Bio-Rad CFX-96, CHKBio Q9600, Thunder Series qPCR, SLAN96p, Molarray MA-6000, iNAT-POC and other real-time fluorescent PCR instruments, etc.

【SAMPLING】

(1) Pharyngeal swab: Wipe bilateral pharyngeal tonsils and posterior pharyngeal wall at the same time with a plastic rod swab with polypropylene fiber head, immerse the swab head into a tube filled with normal saline or VTM solution(Cat No.:VTM001,manufactured by CHKBiotech), discard the tail, and tighten the tube cap. Vortex and use 200uL of the sample for nucleic acid extraction.

(2) Sputum: After rinsing with clean water in the morning, inhale deeply and cough up the second sputum deep in the respiratory tract, and spit it in a clean sputum container. The sample shall not be less than 1ml, and mouthwash and food residues shall not be mixed in sputum.

Add same volume of 4% NaOH to the collected sputum sample and vertex shortly, digest it for 30min, take 1mL of the digested sample to a 1.5mL centrifuge tube and centrifuge at 13000rpm for 5 min, discard the supernatant, add 1mL saline or 1×PBS solution into the tube, centrifuge at 13000rpm for 5 min, discard the supernatant, add 500uL saline or 1×PBS solution into the tube,vertex and use 200uL of the sample for nucleic acid extraction..

(3) Bronchoalveolar lavage fluid(BALF): Correctly collect broncho-alveolar lavage fluid. Take 1mL of the collected sample to a 1.5mL centrifuge tube and centrifuge at 13000rpm for 5 min, discard the supernatant, add 500uL saline or 1×PBS solution into the tube, vertex and use 200uL of the sample for nucleic acid extraction.

(4) Cultured Mycobacterium tuberculosis specimens: Collect culture with inoculating loop and then suspend in 500μL of normal saline or 1×PBS solution; for Mycobacterium tuberculosis cultured on liquid medium, collect 1mL culture and centrifuge at 13000rpm for 5 min, then suspend bacterial pellet in 500μL of normal saline or 1×PBS solution, vertex and use 200uL of the sample for nucleic acid extraction.

(5) Fresh tissue: Cut a fresh tissue specimen of 50 mg and grind it in a sterile vessel. Suspend in 500μL of saline or 1×PBS solution,vertex and use 200uL of the sample for nucleic acid extraction.

The sample can be used for testing immediately, or stored at 2-8°C for no more than 3 days, stored at -20°C for six months, and at -70°C for 2 years. The samples should be transported in cold chain box to avoid repeated freezing and thawing and in accordance with biosafety regulations.

【TESING PROTOCOL】

1. Reagent Preparation

a) Lyophilized in bottle version kit:Add **750uL** dissolving solution to the bottle to dissolve the lyophilized powder of PCR Mix A and PCR Mix B. Divide **15uL** of the dissolved reagent into each PCR reaction tube.

b) Lyophilized 8-well strip version kit: Add **15uL** dissolving solution to each tube well to resolve the PCR Mix A and PCR Mix B.

c) Positive Control :Add **40uL** dissolving solution to one tube well of positive control to resolve it. Shock and centrifuge them at low speed.

d) Internal Control :Add **140uL** dissolving solution to one tube well of internal control to resolve it. Shock and centrifuge them at low speed.

The dissolved reagent and positive control can be temporarily stored at 4℃ for later use.

*Notes:When using the lyophilized in bottle version kit, after dissolving the reagent can be stored at -20℃ and repeated freeze -thaw should be less than 4 times.

2. Treatment of samples

2.1 Direct lysis method:

Take the Nucleic Acid Release Reagent from the kit and balance it to room temperature.

A) **Pharyngeal Swab sample**: Vertex the cell preservation solution (or Virus Transportation Medium) sample collected with Pharyngeal swab and pipette **20uL** the samples into each 0.5mL centrifuge tube. Add **5uL** of Internal control and **20uL** of Nucleic Acid release reagent into each tube, vertex shortly.

B) **BALF(Bronchoalveolar lavage fluid) sample**: Vertex the BALF samples and pipette **20uL** the samples into each 0.5mL centrifuge tube. Add **5uL** of Internal control and **20uL** of Nucleic Acid release reagent into each tube, vertex shortly.

C) Sputum sample:

1）Quick method：Vertex the 4% NaOH Pre-treated sputum specimen and pipette **5uL** the samples into each 0.5mL centrifuge tube. Add **5uL** of Internal control and **45uL** of Nucleic Acid release reagent into each tube, vertex shortly.

2）Standard method： Centrifuge the 4% NaOH Pre-treated sputum specimen for 2 minute at 13,000 rpm. Discard the supernatant and add 1mL 1×TE solution. Vertex for 10 seconds and centrifuge for 2 minute at 13,000 rpm. Discard the supernatant and add 100uL 1×TE solution. Vertex for 10 seconds and pipette **5uL** the samples into each 0.5mL centrifuge tube. Add **5uL** of Internal control and **45uL** of Nucleic Acid release reagent into each tube, vertex shortly.

Note: The 4% NaOH solution and the 1×TE solution need to be prepared by the users.

The total nucleic acid(DNA/RNA) can be released within 5 min after adding the Nucleic Acid Release Reagent and it is ready do PCR test next.

1.2 DNA extraction method: Use the appropriate nucleic acid extraction kit and follow the extraction kit manual to extract nucleic acid from the specimen. Nucleic Acid Extraction Kit (Magnetic Beads)(Cat No.:EX004) of CHKBiotech is recommended. Please note to ddd **5uL** of Internal control and **200uL** of pre-treated liquid sample into the lysis well of the extraction reagent. And then do the nucleic acid extraction process.

After DNA extraction, the nucleic acid template should be added to the PCR reaction tube within 30 minutes, or store them at 2℃~8℃ temporarily(no more than 24hours). For longer storage should be at -20℃±5℃(no more than one month).

3. Template Addition

Add 10uL negative control, 10uL positive control, and **10uL** nucleic acid extracted from each specimen to each PCR reaction tube. Vertex and centrifuge at low speed. Then, place them into the real-time PCR machine.

4. PCR amplification

PCR cycle parameter setting

No	Temperature(℃)	Time	cycle
1	95	2mins	1cycle
2	95	10s	40cycles
3	60	20s	

- Detection channel settings: **In PCR Mix A tube:** TB selects FAM fluorescence channel, NTM selects ROX fluorescence channel, Internal reference selects HEX (VIC) fluorescence channel and MKA selects CY5 fluorescence channel. **In PCR Mix B tube:** MAV selects FAM fluorescence channel, MIN selects HEX(VIC) fluorescence channel, MAB selects ROX fluorescence channel and MMS selects CY5 fluorescence channel.
- For ABI7500 software operation interface select "None" for reference fluorescence。
- Set up fluorescence signal acquisition: the fluorescence signal acquisition is set at 60℃ annealing and extension step.
- Set sample information: Sample information can be set before or after PCR amplification.

5. Data Analysis

The test data file needs to be saved after the PCR reaction. Please set the parameters and analyze the results of the FAM, ROX, HEX and CY5 channels separately. The following analysis principles are universal for all the real-time fluorescent PCR instruments listed in 【**INSTRUMENTS**】chapter.

(1) Baseline setting: The baseline can be set automatically or adjusted according to the shape of the amplification curve.

(2) Threshold setting: the threshold should be higher than the highest fluorescence value of the negative control in the kit.

6. Quality Control

Negative and positive controls provide quality control for the assay and should be performed for each test. The result is valid if all of the following conditions are met. Otherwise, the test is invalid. In this case, errors in instruments, reagents, amplification conditions, etc. should be checked and the experiment repeated.

Product of Quality Control		Requirements of Quality Control			
		FAM Channel	HEX Channel	ROX Channel	CY5 Channel
PCR Mix A	Positive Control	Ct ≤ 32	Ct ≤ 32	Ct ≤ 32	Ct ≤ 32

	Negative Control	Undet	Undet or Ct>38	Undet	Undet
PCR Mix B	Positive Control	Ct ≤ 32	Ct ≤ 32	Ct ≤ 32	Ct ≤ 32
	Negative Control	Undet	Undet	Undet	Undet

7. Interpreting Test Results

PCR Mix A					Interpretation of result
FAM Channel (TB)	HEX Channel (IC)	ROX Channel (NTM)	CY5 Channel (MKA)	Comment	
Ct≤38	Ct≤32	Undet or Ct>35	Undet or Ct>35	-	TB Positive
Undet or Ct>38	Ct≤32	Ct≤35	Any	-	NTM Positive
Ct≤38	Ct≤32	Ct≤35	Any	FAM Ct < ROX Ct	TB Positive
Ct≤38	Ct≤32	Ct≤35	Any	FAM Ct >ROX Ct	TB and NTM both Positive
Undet or Ct>38	Ct≤32	Ct≤35	Ct≤35		MKA Positive
Undet or Ct>38	Ct≤32	Undet or Ct>35	Undet or Ct>35		Negative
Any	Undet or Ct>32	Any	Any		Re-sampling an test again

PCR Mix B				Interpretation of result
FAM Channel (MAV)	HEX Channel (MIN)	ROX Channel (MAB)	CY5 Channel (MMS)	
Ct≤35	Undet or Ct>35	Undet or Ct>35	Undet or Ct>35	MAV Positive
Undet or Ct>35	Ct≤35	Undet or Ct>35	Undet or Ct>35	MIN Positive
Undet or Ct>35	Undet or Ct>35	Ct≤35	Undet or Ct>35	MAB Positive
Undet or Ct>35	Undet or Ct>35	Undet or Ct>35	Ct≤35	MMS Positive

*If the result of Ct of the internal reference (HEX channel of PCR Mix A) is Undet or Ct>32, the test result is invalid and should be resampled and tested again.

【CUT-OFF VALUE OR REFERENCE INTERVAL】

The cut-off values of TB positive is Ct≤38 and the cut-off values of NTM, MAV, MIN, MAB, MMS, MKA positive is Ct≤35.

【ASSAY EXPLAINATION】

(1) Contamination of laboratory environment and reagents, or cross-contamination during specimen processing may lead to false positive results.
(2) If the transportation, storage and operation of the reagent are wrong, it may even lead to false negative results, resulting in a decrease in the detection effect.
(3) Patients with negative Mycobacterium tuberculosis and non-tuberculosis citrus cannot rule out early infection or recovery period. If possible, collection of more sensitive bronchoalveolar lavage fluid samples for retesting is recommended for symptomatic suspected patients.

【ASSAY LIMITATIONS】

1. The positive result of the kit test does not indicate whether there is active TB or NTM in the body. Other confirmation methods are recommended at the same time.
2. This kit is used for the classification and detection of Mycobacterium tuberculosis and non-tuberculosis mycobacteria. This result is for clinical reference only and should be considered for clinical management of the patient in conjunction with the patient's symptoms/signs, medical history, other laboratory tests, and response to treatment.
3. Although the target sequences detected by this kit are the conserved gene regions of Mycobacterium tuberculosis(IS6110 and IS1081 gene) and non-tuberculosis mycobacteria(16S gene), theoretically, the missed detection of TB, NTM, MAV, MIN, MAB, MMS and MKA with rare mutations in the conserved regions can not be completely avoided.

【PERFORMANCE SPECIFICATIONS】

Conformity rate of negative control: 8 enterprise reference samples (T1-T8) tested negative for TB and NTM, and the conformity rate of negative control(-/-) is 100%(8/8).

Conformity rate of positive control: the test result of Mycobacterium tuberculosis and non-tuberculosis mycobacteria was positive. Weak positive detection rate of 95% (n≥20), moderate positive detection rate of 100% and CV≤5% (n≥20)

Limit of detection(LOD): The samples with the minimum detection limit are repeated no less than 20 times, with a positive detection rate of 95%. The LOD of kit is 500 copies/ml or 5 copies/reaction

Repeatability: The enterprise reference sample (J1, J2) was positive after 10 repeated tests, and the coefficient of variation (CV) of the J1 Ct value was less than 5.0%.

Precision: continuous detection for 20 days, 2 times per person per day, 2 repetitions for each sample, the coefficient of variation of the Ct value CV<5.0%.

Specificity (interference): Non-specific interference with other related pathogens Influenza A virus (H1N1, H3N2, Flu-A), Influenza B virus, Streptococcus pneumoniae virus, Escherichia coli virus, Candida albicans, Hepatitis B virus , parainfluenza virus (I, II and III) and Metapneumovirus.

【ATTENTIONS】

1) This kit is only used for auxiliary diagnosis in vitro; the clinical diagnosis and treatment of patients should be comprehensively considered in combination with their symptoms/signs, medical history, other laboratory tests and treatment response;
2) This kit selects the conserved region of TB bacterial gene as the detection target and the normal TB variants of Rifampicin and isoniazid resistance will not affect the detection performance and the kit can detect them.
3) Please read the instructions of this kit carefully before the experiment, and strictly follow the operation steps;
4) Unreasonable sample collection, transport, storage and processing may lead to false test results; if the sample processing process is not controlled, cross-contamination may occur, and false positive results may occur;

【General Information】

Manufacturer name: Shanghai Chuangkun Biotech Inc.

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Tell：0086-21-60296318

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【European Anthorized Representative】

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【Date of Approval and Revision of Instructions】:2021-05-25V7.0