TB/NTM Nucleic Acid Detection Kit (Lyophilized)

Use Manual

Label	Description	Label	Description
	Contains sufficient for <N> tests		In vitro diagnostic
	Batch number		Consult instruction before use
	Date of manufacturing		SUNGO Europe B.V. Olympisch Stadion 24, 1076DE Amsterdam,Netherlands
	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】

48Tests/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 differentiate and diagnose Mycobacterium tuberculosis and Non-tuberculosis mycobacteria infection. 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 . The FAM and ROX channels are used for the qualitative detection of Mycobacterium tuberculosis/Non-tuberculosis mycobacteria pathogen genes respectively, and the HEX channel is used to qualitatively detect the human internal reference gene RNaseP. 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(Lyophilized)	15μl×48	6×0.2ml 8 well-strip tube	Contains primers and fluorescent probes of TB/NTM and internal control, dNTPs, $MgCl_2^+$,Taq DNA polymerase
	750μl	1×bottle	
Dissolving solution	800μl	1×1.5ml tube	DEPC treated purified water,
Negative Control	200μl	1×1.5ml tube	0.9%NaCl
Positive Control	20μl×2	2×0.2ml tube (Lyophilized)	Plasmid of TB/NTM DNA and internal standard

【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, Bio-Rad CFX-96, SLAN96p, Molarray MA-6000 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, discard the tail, and tighten the tube cap.

(2) Sputum: Immerse the sputum or the swab head dipped in the sputum into the test tube filled with normal saline or VTM, and tighten the cap of the tube.

(3) Bronchoalveolar lavage fluid: Correctly collect bronchoalveolar lavage fluid for testing.

Samples collected should be used for testing as soon as possible. If the sample to be transferred can not be detected immediately, keep it cryopreserved. Samples can be stored at 2~8℃for 24 hours, and can be stored for a long time below-70℃. It can also be temporarily stored in a -20℃refrigerator. Samples should be transported at low temperature in accordance with biosafety regulations.

【TESING PROTOCOL】

1. DNA nucleic acid extraction

Extract nucleic acids from specimens using an appropriate nucleic acid extraction kit and following the extraction kit's instructions. After DNA extraction, the extracted DNA should be added to a PCR reaction tube within 60 minutes, or transferred to a centrifuge tube and stored at -15°C ~ -25°C.

2. Reagent Preparation

a) Lyophilized in bottle version kit:Add 750uL dissolving solution to the bottle to dissolve the lyophilized powder. 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.

c) Positive Control :Add 20uL dissolving solution to one tube well of positive 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.

3. PCR amplification

PCR cycle parameter setting

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

A. Detection channel settings: TB selects FAM fluorescence channel, NTM selects ROX fluorescence channel, and internal reference selects HEX (VIC) fluorescence channel. For ABI7500 software operation interface select "None" for reference fluorescence。

B. Set up fluorescence signal acquisition: the fluorescence signal acquisition is set at 60℃ annealing and extension step.

C. Set sample information: Sample information can be set before or after PCR amplification.

4. Data Analysis (ABI7500)

The test data file needs to be saved after the PCR reaction. Please set the parameters and analyze the results of the FAM, ROX and HEX channels separately.

(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.

5. 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	ROX Channel	HEX Channel
Positive Control	Ct ≤ 32	Ct ≤ 32	Ct ≤ 32
Negative Control	Undet	Undet	Undet or Ct>38

6. Interpreting Test Results

Channel			Interpretation of result
FAM Channel	ROX Channel	HEX Channel	
Ct≤38	Ct≤38	Ct≤32	TB and NTM both Positive
Ct≤38	Undet or Ct>38	Ct≤32	TB Positive
Undet or Ct>38	Ct≤38	Ct≤32	NTM Positive
Ctt>38	Ctt>38	Ct≤32	Negative
Any	Any	Undet or Ct>32	Re-sampling an test again

*If the result of Ct of the internal reference (HEX channel) 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 and NTM positive result are Ct≤38.

【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 regions of Mycobacterium tuberculosis and non-tuberculosis mycobacteria genes, theoretically, the missed detection of TB and NTM 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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【European Anthorized Representative】

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【Date of Approval and Revision of Instructions】:2021-05-25V1.1