# Mycobacterium Tuberculosis (TB) Nucleic Acids Detection Kit User Manual (Fluorescent PCR Method)

## 【Product Name】

Generic name: Mycobacterium Tuberculosis (TB) Nucleic Acids Detection Kit

## 【Packing】

48 Tests/kit

## 【Intended Use】

This kit is used for qualitative detection of mycobacterium tuberculosis nucleic acid in sputum specimens using PCR amplification technique. Patients with positive result indicate the mycobacterium tuberculosis infection, but should not be diagnosed as tuberculosis patients. Comprehensive judgment combined with clinical data is necessary.

## 【Detection Principle】

The kit combines PCR technology with fluorescent probe technique to amplify and detect the highly conserved specific nucleic acid sequence of TB in the sputum to determine the presence of TB. Detection sensitivity is $10^1$ TB bacteria/mL. The use of uracil -N- glycosylase and dUTP in the kit can effectively eliminate the interference of the carried-over amplification product contamination on the test results.

## 【Kit Contents】

| Contents | Specification |
|---|---|
| DNA Extraction Buffer | 1.2mL x 2 vials |
| PCR Reaction Solution | 888μL x 2 vials |
| Reaction Enhancer | 384μL x 1 vial |
| Negative Control | 1mL x 1 vial |
| Positive Control | 1mL x 1 vial |
| Critical Positive Control | 1mL x 1 vial |

## 【Storage】

Stored at -20±5℃, away from lights. Avoid being repeatedly frozen and thawed. The validity period is 8 months..

## 【Compatible Instruments】

ABI7500, STRATAGENE Mx3000p PCR instruments etc.

## 【Sample Collection, Storage and Transport】

1. Specimen collection: Collect 3-5mL of the sputum specimen by disposable collector in the morning and seal tight for testing.
2. Samples can be stored at 2-8℃ for 24 hours, -20±5℃ for 3 months, and -70℃ for a long-term storage. Unnecessary repeated freezing/thawing should be avoided.

## 【Procedure】

### 1. Reagent Preparation

Calculate the reaction number N (N = sputum specimen number + negative control + positive control + critical positive control). Pipet N x 37μL PCR reaction solution and N x 8μL Reaction Enhancer into one centrifuge tube and vortex for 5 seconds, and then aliquot them into N x PCR

reaction tubes with 45μL/tube and reserved at 4°C. Transfer the DNA extraction buffer, negative control, positive control and critical positive control into 4 ℃ refrigerator as well.

**2. Sample Processing**

**2.1** Mix same volume of NALC-NaOH with the sputum sample and put at room temperature for more than 40 min.

**2.2 Sputum specimen treatment:** Pipet 1mL sputum diluent solution into 1.5mL centrifuge tube, centrifuge for 10 min at 13000rpm and discard the supernatant. Add 1mL TE solution (onsite prepared) to the precepite, centrifuge for 10 min at 13000rpm and discard the supernatant. Repeat the above step one more time. Add 50μL DNA extraction buffer, vortex and then incubate for 15 min at 100°C and then centrifuge for 10 min at 13000rpm.

**2.3 Treatment of negative control, positive control, and critical positive control:** centrifuge the control samples for 10 min at 13000rpm and discard the supernatant. Add 50μL DNA extracted buffer and incubate for 15 min at 100°C then centrifuge for 10 min at 13000rpm.

**2.4** Add 5μL supernatant of the specimen, negative control, positive control, critical positive control to corresponding PCR reaction tubes and spin down briefly.

**Note:**

**(1)** Preparation of NALC-NaOH: Mix 50mL of 2.94 %( 0.1mol/L) sodium citrate with 50mL of 4% NaOH. Add 0.5g NALC (N-Acetyl-L-cysteine) before use and store at room temperature. Use within 24-48h.

**(2)** Preparation of Tris-EDTA (TE solution): Mix 10mL of 1mol/L Tris-EDTA and 2mL of 0.5mol/L EDTA, and then add purified water to 1000mL.

**3. PCR Amplification**

Proceed with amplification according to below cycle parameters.

Cycle Parameters:

| | | |
|---|---|---|
| Stage 1, | 50°C | ---- 2 min |
| Stage 2, | 94°C | ---- 5 min |
| Stage 3(40 cycles), | 93°C | ---- 20 sec |
| | 60°C | ----60 sec |

Note: collect the fluorescence signal at 60°C, set the fluorescein as FAM, reaction volume 50μL.

**4. Result Analysis**

**4.1** For STRATAGENE Mx3000P PCR instrument: data will be saved after the PCR reaction and use the software to analyze the result automatically.

**4.2** For ABI7500 PCR instrument: set the BASELINE value as 3-15 (baseline cycler can be adjusted from 2-18 accordingly). The threshold setting principle is that the threshold line just exceeds the maximum of the normal negative control amplification curve (irregular noise line) and Ct value=40.0

**5. Quality Control**

This kit provides 1 x negative control, 1 x positive control and 1 x critical positive control and the respective Ct value is Ct negative, Ct positive and Ct critical positive. If the kit is in good quality and test procedure is correct, Ct positive ＜ Ct critical positive ＜ Ct=36, Ct negative=40. Otherwise, the test is invalid and retest is suggested. Include a positive control and a negative control in every single test.

**【Results Interpretation】**

**If the test result is valid,**

| | | |
|---|---|---|
| Ct=40<br>or "No Ct" (Mx3000p)<br>or "Undet" (ABI7500) | Negative | |
| 36≤Ct＜40 | Gray area, retest 2 times | 2 times Ct=40, negative<br>1 out of 2 Ct＜40, positive |
| Ct＜36 with standard curve | Positive | |

**【Kit Performance】**

Detection sensitivity is $10^1$ x TB bacterium/mL.

Accordance rate of positive and negative result is 100%

**【Warnings and Precaution】**

Carefully read this instruction before starting the procedure.

1. For in vitro diagnostic use only. Test should be carried out by skilled personnel.
2. Use calibrated pipettors to ensure the test accuracy and reliability. Use disposable PCR reaction tubes, centrifuge tubes and filters and make sure there are no any DNA or RNA enzymes in these test materials.
3. Strict zoning operation is required for the experiment. All the articles and work clothes in each zone are special for use. They should not be used interchangeably to avoid contamination. Clean the workbench immediately after the experiment.
4. Fully thaw and mix the reagent at room temperature and spin down briefly before use.
5. Specimen preparation should be treated in bio-safety cabinet to protect operator and avoid environmental contamination.
6. Include a positive and a negative control in every experiment. Do not mix use the reagents from different batches and use the kit within its valid date.
7. Marks on the PCR reaction tubes are prohibited in order to avoid exogenous fluorescent signal interference.
8. Fully thaw the frost DNA specimens at room temperature before use and spin them down briefly.
9. Reaction tubes should be covered tightly or seal the reaction tubes in plastic bags before transfer them to the specimen preparation area.
10. When adding DNA samples make sure the specimen is completely droped into the reaction liquid and seal the tube quickly.
11. Avoid bubbles in making reaction aliquots. Ensure the tubes are covered tightly to avoid any leaks causing instrument contamination.
12. Take out the reaction tubes after amplification and seal them in a special plastic bag. Dispose them to the assigned place.
13. Discard the pipetting tips into the waste container with 10% sodium hypochlorite. Dispose them with other wastes after sterilization.
14. After the test, clean the cabinets and pipettor with 10% sodium hypochlorite, 70% ethyl alcohol and ultraviolet radiator.
15. Preheat the real time fluorescent PCR instrument for 30 min before use. If continue with another experiment, reuse the instrument after 1 hour break.

16. Frequently calibrate the real time fluorescent PCR instrument and clean the specimen plate.
17. Positive control from this kit and the detected specimens may be biohazardous and should be treated according to medical waste standards.

**【Manufacturer】**

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