Mycobacterium tuberculosis PCR Detection Kit

Cat. No.: PK3071
Storage: -20°C

Quantity: 50 Reactions
Shipment: Wet Ice

This kit is designed for qualitative detection of Mycobacterium tuberculosis (MTB) DNA in the Human sample by the method of Polymerase Chain Reaction.

Kit Contents:

The kit for 50 amplification reactions consists of:

1. 1x PCR MIX	**1000µl**
2.Taq DNA polymerase	**20µl(5u/µl)**
3. Mineral Oil	**2ml**
4. DNase Free, Deionized Sterile Water	**5ml**
5. Positive Control	**50µl (1pg/µl)**
6. TB Lysis Solution	**5ml**

The **Kit** should be stored at **–20°C**.

Sample preparation:

Performed in Pre-amplification, specimen, and control preparation area.

For DNA extraction from clinical samples use SinaClon **DNP™Kit (Cat.No.:EX6071)** or **SinaPure™ DNA Extraction Kit (Cat No.:EX6021).** DNA can also be extracted by other standard methods like Phenol/Chloroform.

For DNA extraction from culture media, resuspend one colony of MTB bacteria in 200µl **TB Lysis Solution** and incubate 30 min at 95°C then centrifuge 15 min. at 10000 RPM and store supernatant (containing DNA) at –20°C.

PCR Protocol :

Performed in pre-amplification, Reagents preparation area.

1. Take out the kit and unfreeze the tubes, then put all the tubes on ice. Vortex and spin tubes before opening. The final volume of each PCR reaction will be 25µl.

2. Label new 0.5 tubes for amplification reaction(s) for test(s), positive and negative control.

3. Add the following reagents for each tube on ice:

1x PCR MIX	**20µl**
Taq-DNA polymerase	**0.4µl**

Note: To avoid contamination all reagents must be taken with separate clean tips!

4. Mix the mixture thoroughly by shaking and spin.
5. To each tube add one-drop (20-25µl) **mineral oil.**
Note: In this step, cap the reaction tubes or place the tube try in resalable plastic bag and seal the bag securely, don't cap tubes at this time. Do the next steps on Pre-amplification, specimen, and control preparation area.

6. Add **5µl DNA** (Use specified pipette for sampling of DNA).
7. Close tubes; spin the mixtures on microfuge for 3-5 sec.

8. Transfer the tubes to preheated thermocycler and start the program:

Cycling parameters:

93°C-60sec		93°C-20sec
72°C-50 sec	**Follow by =>**	72°C-50sec
1 cycle		**37cycle**
		93°C-20sec
	End by =>	72°C-120sec
		1 cycle

Results Analysis :

Performed in Post-amplification, area

Load 10µl amplification samples directly in a 2% agarose gel without adding loading buffer. The presence of **163 bp** fragments comparing with DNA size marker indicates positive test.

For Research Use Only

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