SimPCR™ MTB Detection Kit

(Mycobacterium tuberculosis)

Cat.No: SBL12-2210

USER MANUAL

INTRODUCTION

Mycobacterium tuberculosis is a species of pathogenic bacteria in the family Mycobacteriaceae and the causative agent of tuberculosis.

The physiology of M. tuberculosis is highly aerobic and requires high levels of oxygen. Primarily a pathogen of the mammalian respiratory system, it infects the lungs. The most frequently used diagnostic method for tuberculosis is the polymerase chain reaction (PCR).

The SimPCR™ Mycobacterium tuberculosis Detection Kit is designed for Mycobacterium tuberculosis detection by the Polymerase Chain Reaction (PCR) method.

KIT CONTENTS

Components	Labels	Cap Color	Volume 50 Tests
2x Reaction Mix	Reaction Mix	Black	500 µl
Primer & Probes	Oligomix	White	100 µl
TB Positive Control	Control	Green	30 µl

TEST PRINCIPLE

TB detection is based on the amplification of the pathogen genome specific region using the TB specific primers. The product amplification has been confirmed by agarose gel electrophoresis.

PROTOCOL

a) Preparation of the PCR mix

For each experiment, prepare a master mix of an appropriate volume for negative control, positive sample, and (n+1) test samples. The reagents have to mix under this ratio:

Component Labels	Volume/reaction
Reaction Mix	10 µl
Oligomix	2 µl

NOTE: *For each PCR set, prepare one positive and one negative controls.*

Aliquot 12µl of Master Mix in each tube and add 2-4µl of extracted DNA or control DNA into individual tubes; then, add DW up to 20µl; spin tubes shortly and place them in your thermocycler device.

b) Thermal cycler programming

Set device as the following profile:

Step	Temp	Time	Cycle
Initial activation	95˚C	5 min	1 X
Denaturation	95˚C	30 s	40 X
Annealing	60˚C	30 s	
Extension	72˚C	30 s	
Final Extension	72˚C	5 min	1 X

Notice: appropriate reaction template files can be found in www.sim-biolab.com

DATA ANALYSIS

Run 5µL of PCR products alongside a DNA marker (100bp) on a 2% agarose gel. The TB render a band of approximately 300bp.

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