

Mycobacterium Tuberculosis Detection Protocol

A. Introduction

This protocol uses end point PCR[†] with BHQ_{plus}[™] probes to test for *Mycobacterium tuberculosis* (MTB). The target sequence is the *IS6110* insert element, which is specific to human strains of MTB.

PCR run time is 35 min.

B. Equipment

- Spartan DX[™] instrument
- Microcentrifuge
- Vortex
- Ice bucket or cold block
- Pipettes

C. Materials

- 0.2 ml flat-cap PCR tubes (VWR, Cat. No. 53550-106)
- Filtered pipette tips
- PCR-grade Mineral Oil (Biotools, Cat. No. 20.032)
- BIOTUB-QT kit (Biotools, Cat. No. 90.572C)
- Sterile water (DNase-free)
- 1.5 ml microcentrifuge tubes
- Primers that recognize the *IS6110* insert element (Integrated DNA Technologies)(Table 1)
- BHQ_{plus} probe (Biosearch Technologies) that recognizes the *IS6110* insert element (Table 1)

Primer/Probe	Forward (5'-3')	Reverse (5'-3')	Amplicon size (bp)
<i>IS6110</i> primers	ATC TCg TCC AgC gCC gCT TC	CCC TgC CCA ggT CgA CAC AT	83
<i>IS6110</i> probe	6FAM-CCA CAg CCg gTT Agg T-BHQ _{plus}		

6FAM = 6-carboxy-fluorescein, BHQ_{plus} = Black Hole Quencher *plus*

Table 1. Primer/probe sequences and amplicon size.

D. Preparation

1. Any commercially-available DNA purification kit may be used
2. Turn on Spartan DX instrument and let it warm up for a minimum of 10 min
3. Set up thermal cycling program as per Table 2

Step	Temperature	Time	Cycles
Initial denaturation	97.0°C	12 s	1
Denaturation	97.0°C	24 s	50
Annealing/extension	45.0°C	16 s	50

Table 2. Cycling parameters.

E. Protocol

1. Prepare a master mix for *IS6110* as per Table 3
2. To test one unknown sample, we recommend setting up reactions as follows:
 - Tube 1: 15 µl of *IS6110* master mix
 - Tube 2: 15 µl of *IS6110* master mix
 - Tube 3: 15 µl of *IS6110* master mix
 - Tube 4: 15 µl of *IS6110* master mix
3. In a separate lab area, add the following to each tube:
 - Tube 1: 2.5 µl of purified DNA (sample in question) + 2.5 µl of sterile water
 - Tube 2: 2.5 µl of known MTB-positive DNA + 2.5 µl of sterile water (positive control)
 - Tube 3: 2.5 µl of purified DNA (sample in question) + 2.5 µl of known MTB-positive DNA (purification control)
 - Tube 4: 5 µl of sterile water (negative control)
4. Mix and spin down reactions
5. Overlay reaction mixture with 15 µl of mineral oil
6. Spin down reaction tubes
7. Insert samples into Spartan DX instrument and start your run
8. End point PCR is determined to be positive if the last cycle reading has a fluorescence value greater than 5
9. Example of real-time graph is depicted in Figure 1

Reagent	IS6110 Master Mix	
	Reaction Formulation	Volume
QUANTIPROBES Reaction Mix (2X)	10 µl x (____+0.5*) Sample #	
Forward primer (10 µM)	1 µl x (____+0.5*) Sample #	
Reverse primer (10 µM)	1 µl x (____+0.5*) Sample #	
Probe (1 µM)	2 µl x (____+0.5*) Sample #	
Sterile water	1 µl x (____+0.5*) Sample #	
Total volume of master mix	15 µl/reaction	____ µl

* Recommended volume correction factor for pipetting error.

Table 3. Components of PCR master mix for MTB.

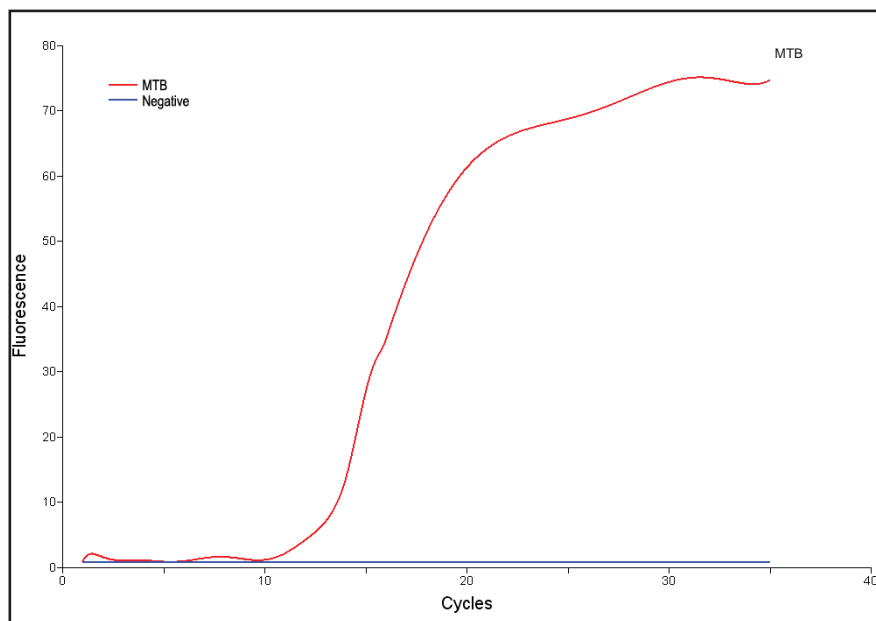


Figure 1. Real-time PCR result for *M. tuberculosis* (MTB).

† - An end point assay is described as an assay that uses data from images collected at the first and last cycles of a PCR run to determine the success or failure of the reaction. End point analysis mode is selected in the options menu of the SpartanDX™.

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