Quantiplus® MTB FAST Detection Kit (Real-Time Qualitative PCR Kit)

QLF-MTB-25 : 25 rxns
QLF-MTB-50 : 50 rxns
QLF-MTB-100 : 100 rxns

RUO

PI/QLFMTB-02

Introduction

Mycobacterium tuberculosis cause chronic lung infections, only tuberculosis (TB) spreads from person to person by inhalation of organisms expectorated into the air.

Product Description

Quantiplus® MTB FAST Detection Kit is a Real Time PCR based *in-vitro* diagnostic assay for detection of MTB Complex (MTBC) in TB Culture, Body fluids (CSF, Pleural Fluid, Ascetic Fluid and Synovial fluid) Sputum, Pus, Menstrual fluid, Urine and Tissue. The kit contains an advanced formulated qPCR mix with UDG/UNG which enables Performance of **Fast PCR in shorter run time**, UDG/UNG helps in controlling the PCR carryover contamination, Primer probe mix, positive control and internal control.

Kit components

Color Coding (Caps)	Contents	Description	25 rxns (QLF-MTB-25)	50 rxns (QLF-MTB-50)	100 rxns (QLF-MTB-100)
Blue	DNA Fast qPCR Mix with UDG/UNG (2X)	PCR Amplification Mix	1 x 325µL	1 x 650µL	2 x 650µL
Amber	MTB Fast PPM	Target specific Primer probe mix	1 x 50µL	1 x 100µL	2 x 100µL
Natural	IC-B Mix	Exogenous Internal Control-B mix	1 x 300 µL	1 x 600 µL	1 x 1.2mL
Red	MTBFPC	MTB Fast Positive Control	1 x 100 µL	1 x 100 µL	1 x 200 µL
White	MBGPW	Purified water	1 x 500 µL	1 x 500 µL	1 x 1mL

Storage and Transportation Conditions

The kits could be transported at temperature below -20 °C. The kit will remain stable until the expiry date printed on the package, if the storage temperature is kept (-20 ± 5 °C). Kit is stable after 4 repeated freezing/thawing cycles. The reagents should be frozen in aliquots, if they are to be used intermittently.

Technical Specification

Target Sequence	DNA conservative sequence of IS 1081
Specificity MTB genotype	Mycobacterium tuberculosis, M.bovis, M.microti, M.africanum, M.pinnipedii, M. caprae, and M.canettii.
Sensitivity	5 Bacilli
Validated Specimen	TB Culture, Body fluids (CSF, Pleural Fluid, Ascetic Fluid and Synovial fluid), Sputum, Pus, Menstrual fluid, Urine and Tissue.
External Quality Assessment	QCMD EQA Panels

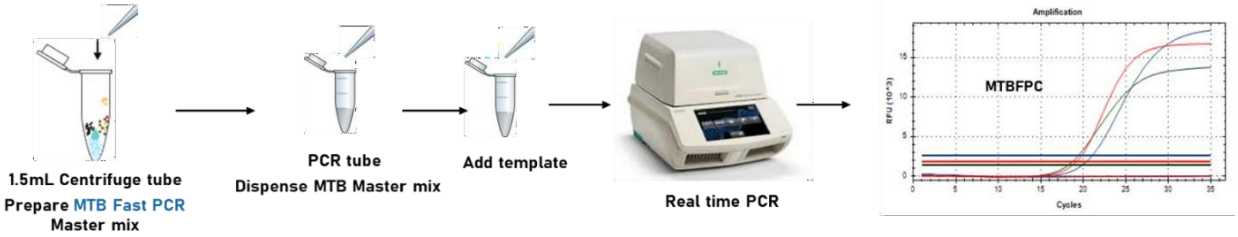
Assay Procedure

DNA Extraction

Quantiplus® MTB FAST Detection Kit has been validated using the following DNA extraction kits:
Recommended sample volume for extraction and elution are as follows:

S. No.	Name of the Extraction Kit	Recommended Sample volume *for Extraction*	Recommended Final Elution volume
1.	QIAamp DNA Blood Mini Kit (Cat. No. 51104)	200 µL	100 µL

Note: Customer can also validate their own extraction process using other DNA extraction Kits.
Contact Huwel for sample type specific protocol



qPCR Protocol Flow

Preparation of Reaction Master mix

Components	Volume per reaction (For 26µL)
DNA Fast qPCR Mix with UDG/UNG (2X)	13.0
MTB Fast PPM	2.0
IC-B Mix (if not added at extraction step)	1.0
Extracted DNA/ MTBFPC / MBGPW	10.0

It is necessary to keep all components at +2 °C to +8 °C during the PCR preparation. Close the tubes, centrifuge shortly before processing in to thermal cycler.

Cycling Conditions

Steps	No. of cycles	Temperature (°C)	Time
1 (Initial denaturation)	1	95	1 min.
2 (PCR cycling)	45	95	10 sec.
		60*	10 sec.
*Plate Read/Data Acquisition in FAM and Texas Red Channel			

Note: Contact Huwel for template for Real Time PCR Program

Sample analysis and Interpretation

The criteria for the acceptance of the assay should be met before the interpretation of the unknown sample results as described in Table 1 below. Interpret the results for unknown samples as mentioned in Table 2.

Table 1

Control	FAM (MTB)	TEXAS RED (IC)
If Internal Control (IC-B Mix) is added during extraction		
Positive Control (PC)	√	-
Negative Control (NC)	-	-
If Internal Control (IC-B Mix) is added during preparation of reaction master mix		
Positive Control (PC)	√	√
Negative Control (NC)	-	√

Table 2

S.No	FAM (MTB)	TEXAS RED (IC)	Fluorophore ← / Interpretation ↓	Conclusion
1	√	√	MTB DNA detected	Proceed for further Analysis
2	√	-		
3	-	√	MTB DNA not detected	
4	-	-	Possible inhibition of PCR	Dilute the DNA sample (1:10) and repeat the Assay

Validated Instruments

- Thermo QS5 Real-Time PCR System
- Bio-Rad™ CFX 96

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