

Quantiplus® MTB/NTM Real Time Qualitative PCR Kit



QL-MTB/NTM-25 : 25 rxns
 QL-MTB/NTM-50 : 50 rxns
 QL-MTB/NTM-100 : 100 rxns

RUO

PI/QLMTB/NTM-04

Intended Use

Quantiplus® MTB/NTM Real-Time Qualitative PCR Kit is used to detect *Mycobacterium tuberculosis* (MTB) complex (*Mycobacterium africanum*, *Mycobacterium bovis*, *Mycobacterium canettii*, *Mycobacterium microti*, *Mycobacterium tuberculosis*) and *Mycobacterium* genus which helps in identifying NTM (Non-tubercular mycobacterium) in pulmonary and extra pulmonary samples. The kit contains amplification mix along with MTB, NTM, Internal Control Primers and Probes Mix (PPM), Internal Control (IC-B mix), Huwel MTB/NTM PC along with Huwel PW.

Background Information

Patients seeking treatment with respiratory specimens positive for acid-fast bacilli present dilemma. Although *Mycobacterium tuberculosis* and non-tuberculous mycobacteria (NTM) cause chronic lung infections, only tuberculosis (TB) spreads from person to person by inhalation of organisms expectorated into the air. NTM infections are mostly acquired from the environment, where they are often present in soil and water sources. As definitive identification of mycobacterial species can take several weeks, the ability to quickly distinguish NTM from TB is useful. In immunocompetent hosts NTM primarily causes respiratory infections, especially in individuals with chronic obstructive pulmonary disease and may cause pulmonary fibrosis or cavitary lung disease, while in immunocompromised hosts it produces systemic bacterial infection. Patients with MTB infection are easily and successfully treated with primary antituberculosis drugs, while the therapy of the disease caused by NTM is often long, strenuous, and not always successful. Thus, it is important to differentiate infections due to MTB and NTM at the early stage of the disease. Real-Time PCR is a quick and sensitive way to detect MTB and NTM.

Kit Components

Color Coding (Caps)	Contents	Description	25 rxns (QL-MTB/NTM-25)	50 rxns (QL-MTB/NTM-50)	100 rxns (QL-MTB/NTM-100)
Amber	Huwel MTB/NTM Ready Mix	MTB/NTM and internal control, Probes and primers with amplification Mix	1 x 375 µL	1 x 750 µL	2 x 750 µL
Natural	Huwel IC-B Mix	Internal Control	1 x 300 µL	1 x 600 µL	2 x 600 µL
Red	Huwel MTB/NTM PC	MTB/NTM Positive Control	1 x 100 µL	1 x 100 µL	2 x 100 µL
White	Huwel PW	Purified water	1 x 500 µL	1 x 500 µL	2 x 500 µL

Note: Please pay attention to the cap color coding and the tube contents.

Huwel PW: Molecular Biology Grade Purified Water

Storage and Transportation Conditions

The kits should be transported at temperature below -20 °C. The kit is stable until the expiry date mentioned on the package, if the storage temperature is within -20 ±5 °C. More than 4X freezing and thawing cycles reduces the assay sensitivity. For intermittent usage the reagents should be frozen in aliquots.

Technical specifications

Target Sequence	MTB- IS 1081 and <i>Mycobacterium</i> genus- 16S rDNA
Specificity	100%
Sensitivity	10 Copies /Reaction
Reporting Units	Detected/Not detected
Validated Specimen	Sputum, Body fluids, Tissue samples
External Quality Assessment	TB Culture, Blood- EDTA, Body fluids (CSF, Pleural Fluid, Ascitic Fluid and Synovial fluid) Sputum, Pus, Menstrual fluid, Urine and Tissue, BAL

Assay Procedure

DNA Extraction

Quantiplus® MTB/NTM Real Time Qualitative PCR Kit has been validated using the DNA extraction kits mentioned below.

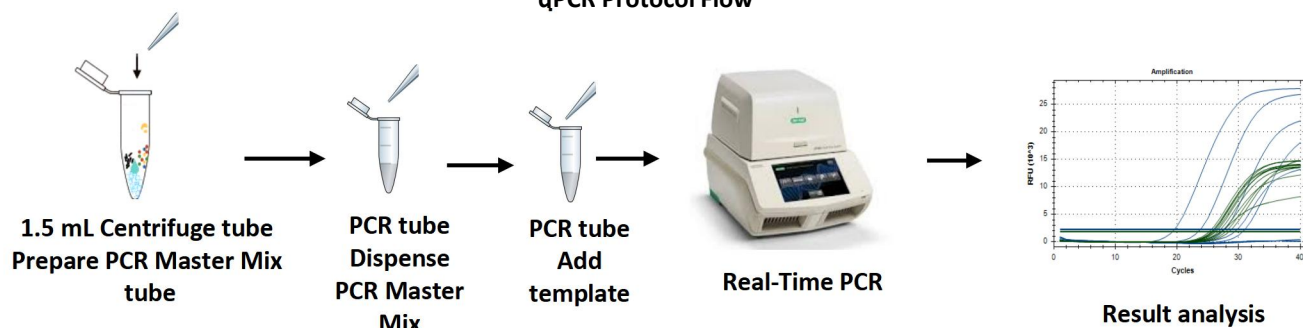
Recommended sample volume for extraction and elution are as follows:

S. No.	Name of the Extraction Kit	Recommended Sample volume for Extraction	Recommended Final Elution volume
1.	Huwel Nucleic Acid Extraction Kit - Version 2.0 (Cat. No. HL-NAX-100)	200 µL	100 µL
2.	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.

Huwel IC-B mix can be added at the extraction step or while setting up the PCR

qPCR Protocol Flow



Preparation of Reaction Master mix

Components	Volume per reaction (for 26µL)
Huwel MTB/NTM Ready Mix	15.0
Huwel IC-B Mix	1.0
Extracted DNA/ Huwel MTB/NTM PC / Huwel PW	10.0

It is necessary to keep all components at +2 °C to +8 °C during the PCR preparation. Close the tubes and centrifuge briefly before proceeding to the thermal cycler.

Cycling Conditions

Steps	No. of cycles	Temperature (°C)	Time
1 (Initial Denaturation)	1	95	15 min.
2 (PCR cycling)	40	95	15 sec.
		58*	60 sec.

*Plate read/Data acquisition in **FAM, Yakima Yellow/VIC/HEX, and Texas Red** channels in Bio-Rad™ CFX 96. For Thermo Q55 Real-Time PCR System, use **FAM, Yakima Yellow/VIC/HEX, and ROX** channels.

Sample analysis and Interpretation

Interpret the values for unknown samples based on the observations as described in the following table and there should be no amplification in the FAM and Yakima Yellow/VIC/HEX channel for negative control. The Ct values of ≤38 Ct for MTBC and ≤34 Ct for NTM of Unknown samples should be considered for positive sample interpretation.

S.No	FAM (MTBC)	Yakima Yellow/VIC /HEX (NTM)	Texas Red/ROX (Internal Control)	Fluorophore
				Interpretation
1	√	√	√	MTBC (Mycobacterium Complex) Detected
2	√	√	-	
3	√	-	√	
4	-	√	√	NTM/ Mycobacterium Genus Detected
5	-	√	-	
6	-	-	√	MTBC/NTM Not Detected
7	X	X	X	Result Not Valid

Note: All the Target channels (FAM, Yakima Yellow/VIC/HEX and Texas Red/ROX) to be analyzed individually.

Validated Instruments

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



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