Mycobacterium tuberculosis [MTB] Real-time PCR Kit

(For Qualitative Detection)

IVD -25°C -15°C

REF 8201

INTENDED USE

MTB Real-time PCR Kit is an in vitro nucleic acid amplification kit for the detection and quantification of Mycobacterium tuberculosis [MTB complex] specific DNA.

KIT CONTENTS

Components	Number of vials	Volume Per vials
Probe PCR Master Mix	1	250µl
MTB Primer Probe Mix [MTB PP mix]	1	65µl
Internal control Primer Probe Mix [IC PP Mix]	1	65µl
Internal control template [IC template]	1	125µl
MTB Positive control [QS1]	1	250µl
Water, PCR grade	1	4ml

STORAGE

1. The kit is shipped on gel ice. Upon arrival, all components should be stored in -20°C. They are stable until the expiration date stated on the label.
2. Repeated thawing and freezing should be avoided, as this might affect the performance of the assay.
3. If the reagents are to be used only intermittently, they should be frozen in aliquots. Storage at 2 to 8°C should not exceed a period of 5 hours.

PRODUCT DESCRIPTION

MTB Real-time PCR Kit constitutes a ready-to-use system for the detection of MTB specific DNA using polymerase chain reaction (PCR). It contains reagents and enzymes for the specific amplification of the conserved region of the MTB genome, and for the direct detection of the specific amplicon in FAM channel. In addition, it contains an internal control amplification system to identify possible PCR inhibition and DNA purification efficiency. External positive control is supplied, which can be used as both qualitative and quantitative to determination the amount of pathogen.

SPECIFICITY

MTB primer and probe have been designed for the specific and exclusive in vitro quantification of MTB Complex [Mycobacterium tuberculosis, Mycobacterium africanum, Mycobacterium bovis, Mycobacterium canettii, Mycobacterium microti.]. The target sequence [MBP64] is highly conserved and has previously been shown to be a good genetic marker for MTB. The primers and probe sequences in this kit have 100% homology with a broad range of clinically relevant reference sequences based on a comprehensive bioinformatics analysis.

ANALYTICAL SENSITIVITY

The analytical sensitivity is defined as the concentration of DNA molecules (copies/µl) that can be detected with a positivity rate of 95%. The analytical sensitivity was determined by analysis of dilution series of quantified MTB specific DNA from 0.001copies to 10copies/µl in triplicates. Under optimal PCR conditions, the analytical sensitivity is 0.45 copies per micro liter.

RNA PURIFICATION

If you are using a spin column-based sample preparation procedure having washing buffers containing ethanol, it is highly recommended to perform an additional centrifugation step for 10 min at approximately 17000 x g (~ 13000 rpm), using a new collection tube, prior to the elution of the DNA.

INTERNAL CONTROL TEMPLATE

Add 5µl of the internal control template to each test sample. Do not add directly to test sample. Add after adding lysis buffer to the test sample [sample/lysis buffer mix]. Complete purification according to the manufacturer's protocols.

Amplification of the internal control template does not interfere with detection of the pathogen target gene even when present at low copy number. The Internal control is detected through the HEX channel and gives a CT value of 21 +/-10.

PREPARATION OF STANDARD CURVE DILUTION SERIES:

1. Pipette 90µl of PCR grade water into three 1.5ml micro centrifuge tubes and label as QS2 to QS4.
2. Pipette 10µl of Positive control-QS1 into tube QS2.
3. Vortex thoroughly and spin down briefly.
4. Change pipette tip and pipette 10µl from tube QS2 into tube QS3.
5. Vortex thoroughly and spin down briefly.
6. Repeat steps 4 and 5 to complete the dilution series.
7. Use 10µl per reaction.
8. Prepare positive control every time fresh.

Standards	Copies per µl
QS-1	200000
QS-2	20000
QS-3	2000
QS-4	200

DETECTION PROTOCOL

- Before use, all kit components need to be thawed completely, mixed by gently inverting and centrifuged briefly.
- Make sure that Positive and Negative control is included in every run.
- Make sure that internal control template is added during DNA purification. If not, pipette 2.5µl of the internal control template directly into the purified DNA.
- Include 0.5 reaction volume for pipetting error while calculating the volume for total number of reactions

Components	Volume per reaction
Probe PCR Master Mix	10µl
MTB PP Mix	2.5µl
IC PP Mix	2.5µl
Master Mix volume	15µl
Purified DNA	10µl
Final reaction volume	25µl

Negative Control setup [NTC]
Add 10µl of PCR grade water.

Qualitative Positive Control setup
Add 10µl of any one of the Positive controls [From QS1 to QS4]

Quantitative Positive controls setup
10µl of all Positive controls prepared from QS1 to QS4.

PROGRAMMING THERMAL CYCLER

Sample volume	25µl
Fluorescence Dyes	FAM & HEX
Passive reference	None
Ramping rate	Default

Cycles	Step	Time	Temp
1	Taq enzyme activation / Hold	15min	95ºC
45 cycles	Denaturation	20sec	95ºC
	Annealing/Data collection*	20sec**	56ºC
	Extension	20sec	72ºC

Data collection/Acquisition	Targets
FAM	MTB complex
HEX	Internal control

** Some qPCR machines may require minimum 30sec for data collection; in that case, set to 30sec, this will not affect the performance.

READING THE GRAPH

Step-1 – Internal control Validation
Select the test samples alone for the internal control analysis. Select HEX dye and view the graph of internal control amplification. A successful amplification Ct value must be within Ct 21 +/- 10.

This range indicates NO PCR inhibition in the reaction. Any sample value goes beyond Ct 31 indicates that either sample has some issues in the purification or inhibiting PCR reaction.

Internal control will not get amplified in the negative and positive controls. Ignore a late noise HEX amplification graph in the NTC and Positive control wells.

Step-2 – FAM - Negative and Positive control validation
Select the NTC and Positive control [Qualitative] or Standards wells [Quantitative], select FAM channel, and view the graph of amplification.

The NTC must be flat with no Ct value. If required adjust the threshold value just above the NTC. The PC or Standards must be amplified as per their copy numbers.

NTC justifies NO contamination in the reagent as well as fine pipetting and its environment. PC justifies the reagents storage conditions and reaction parameters are as prescribed.

Step-3 –FAM - Test Sample status
In FAM channel, select test sample well one by one, analyze the graph/amplification.

QUALITATIVE INTERPRETATION OF RESULTS

Test Sample	Negative Control	Positive Control	Internal Control	Interpretation
Positive	Negative	Positive	Positive	MTB specific DNA detected
Negative	Negative	Positive	Positive	No MTB specific DNA Detected. Sample does not contain detectable amounts of MTB specific DNA.
Negative	Negative	Negative	Negative	Experiment fail
Positive	Positive	Positive	Positive	Experiment fail

Qualitative

Observation		Interpretation
FAM-MTB	FAM-MTB	
<37	<31	MTB DNA Detected

Recommendation

In FAM [MTB] channel, the Ct value beyond 35 is required careful analysis. The analysis may include that the status of NTC amplification curve, threshold adjustment, liner/log scale view assessment, etc.,

Standard curve analysis

1. Interpret the values for unknown samples, only if the R=0.98
2. Slope of calibration is between -3.0 to -3.7.
3. PCR efficiency is between 85% and no amplification in FAM channel of negative control.

Calculating copies or IU per ml

Input the machine indicated copy number into the following formula

$$\text{Result (copies/ml)} = \frac{\text{Result (copies/µl) x Elution Volume (µl)}}{\text{Sample Volume (ml)}}$$

Note:

Elution volume: must be typed in micro liter format, example 30µl, 60µl or 100µl. **Sample volume:** must be typed in milliliter format, example 0.2ml or 0.5ml

Observation	Interpretation
<150copies/ml	MTB DNA detected, but below limit of detection.
150copies to 6 x 10^8	MTB DNA detected and quantified.
< 6 x 10^8	MTB DNA detected, but upper limit of quantitation.

LIMITATIONS

A false negative result may occur if inadequate numbers of organisms are present in the sample due to improper collection, transport or handling. Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test.

The presence of PCR inhibitors may cause under quantification, false negative or invalid results.

Potential mutations within the target regions of the pathogen's genome covered by the primers and/or probes used in the kit may result in under quantification and/or failure to detect.

As with any diagnostic test, MTB Real-time PCR results need to be interpreted in consideration of all clinical and laboratory findings.

QUALITY CONTROL

In accordance with in house Quality Management System, each lot of MTB Real-time PCR kit is tested against predetermined specifications to ensure consistent product quality.

Index of symbols

Consult instructions for use	LOT Batch code	Tests per kit
IVD For *in vitro* diagnostic use only	Do not re-use	Keep dry
2°C 8°C Temperature limit - 25 °C to - 15 °C	Date of manufacture	Biologial Risks
Manufacturer	Use-by date	

Marketed by:

Athenese-Dx Pvt. Ltd.

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