A Real-Time PCR (qPCR) Assay for Detection of the Mycobacterium Tuberculosis Complex

Product Insert

*ab*TES™ MTB qPCR I Kit
Kit version: 1.0

CE IVD

REF 300191 (50 Reactions)
300192 (100 Reactions)

Store at -25°C to -15°C

AITbiotech Pte Ltd
25 Pandan Crescent #05-15
TIC TECH Centre
Singapore 128477

EC REP SIA "Medevice Group"
Jurmalas gatve 32, Riga,
LV-1083, Latvia.
www.medevice-group.com

AITbiotech
Advanced • Innovative • Trusted

For use on Bio-Rad CFX96, Stratagene Mx3005P, ABI 7500 Fast and Qiagen RG Q

1. Pathogen Information

Tuberculosis (TB) is a deadly infectious disease caused by various strains of mycobacteria, usually *Mycobacterium tuberculosis* (MTB) in humans. Worldwide, the incidence of TB is about eight million and about three million people die each year. TB usually attacks the lungs but can also affect other parts of the body. It is spread through the air, when people who have the disease cough, sneeze, or spit. Of the members of MTB complex, *M. tuberculosis* and *M. africanum* are pathogenic in humans only. *M. bovis, M. microti* and *M. pinnipedii*, though etiologic agents of tuberculosis in cows, voles, and seals respectively, are known to infect TB in humans as well.

2. Test Description

The *ab*TES™ MTB qPCR I Kit is a real-time polymerase chain reaction (qPCR) kit for the detection of MTB complex (*M. tuberculosis, M. africanum, M. bovis, M. bovis BCG, M. microti, and M. pinnipedii).* The kit contains all the necessary PCR reagents for rapid, sensitive and reproducible real-time detection of the various members of MTB complex using highly specific primer pairs and double-dye hydrolysis probes. The recommended human sample types are sputum and cerebrospinal fluid (CSF). An **Internal Control (IC)** is also supplied to check for possible PCR inhibition.

3. Storage Conditions

The components of *ab*TES™ MTB qPCR I Kit should be stored in the dark, at -20 °C in a **NON**-frost-free freezer. Frost-free freezers go through freeze-thaw cycles to remain frost-free and may cause accelerated degradation of enzymes and nucleic acids. Avoid repeated thawing and freezing (max 3 times) as this may lower the sensitivity. If reagents will be used intermittently, it is suggested to keep the reagents frozen in aliquots.

4. Kit Components

Tubes	Items	300191 (50 rxns)	300192 (100 rxns)
1	2x Enzyme/Reaction Mix	1x 625 µL	2 x 625 µL
2	Primer Mix	1x 25 µL	2 x 25 µL
3	Probe Mix	1x 25 µL	2 x 25 µL
4	Internal Control	1x 200 µL	2 x 200 µL
5	MTB Positive Control	1x 100 µL	1x 100 µL
6	Nuclease-free Water	1x 600 µL	2 x 600 µL

5. Additional Materials Required but not Provided

- Disposable powder-free gloves
- Nucleic acid extraction Kit
- Vortex mixer
- Pipettes and pipette tips with filter
- Desktop centrifuge with rotor
- Real-time thermal cycler
- 0.2 mL PCR tubes/ 96-well PCR plates
- Ice box/ cooling block

6. Limitations and General Precautions

- The use of this product and its data interpretation are intended for personnel trained in real-time PCR techniques and *in vitro* diagnostics procedures only.
- It is advisable to analyze the real time PCR graph at the end of the run to determine the validity of the Ct data.
- Appropriate specimen collection, transport, storage and nucleic acid extraction procedures are required for reliable results.
- Wear disposable gloves, laboratory coats and eye protection when handling samples and reagents. Wash hands thoroughly thereafter.
- Use sterile pipette tips with filters and replace the tip for every procedure.
- Store and extract positive materials (specimens, controls and amplicons) separately from all other reagents and add to the reaction mix in a separate facility, if possible.
- Thaw all the components thoroughly at room temperature before starting an assay.
- When the components are thawed, mix the components and centrifuge briefly.
- Do not use the kit after its expiration date.

7. Procedures

7.1. Nucleic Acids (NA) Extraction

Standard NA extraction kits are compatible with this assay. Please carry out NA extraction as per instructed in the manufacturer's Extraction Kit manual.

7.2. Internal Control

The supplied IC allows the user to control both NA extraction procedure and to check for possible PCR inhibition. For this application, add 0.4 µL of IC for every 10 µL of elution volume at the beginning of sample preparation. For example, if the extraction protocol uses an elution volume of 50 µL, 2 µL of IC should be added to the sample after the lysis step.

7.3. PCR Reaction Setup

Thoroughly thaw all components, mix and spin briefly. Keep all components and samples on ice. Prepare your PCR reaction based on the following pipetting scheme:

Reagent	Volume per reaction			
	Test sample reaction	Positive control reaction	Negative control reaction	Non template reaction
2x Enzyme/Reaction Mix	12.5 µL	12.5 µL	12.5 µL	12.5 µL
Primer Mix	0.5 µL	0.5 µL	0.5 µL	0.5 µL
Probe Mix	0.5 µL	0.5 µL	0.5 µL	0.5 µL
Nuclease-Free Water	6.3 µL	6.3 µL	11.3 µL	11.5 µL
Internal Control	0.2 µL	0.2 µL	0.2 µL	-
Positive Control	-	5.0 µL	-	-
Extracted Test Sample	5.0 µL	-	-	-
Total Volume	**25µL**	**25 µL**	**25 µL**	**25 µL**

7.4. PCR Cycling Conditions

The following cycling conditions were established and validated on Bio-Rad CFX96, ABI 7500 Fast and Qiagen Rotor-Gene Q. You may need to adjust these conditions for other real-time platforms. **FAM/Green** (MTB) and **Texas Red/Orange** (IC) channels should be chosen and the fluorescence is measured at the annealing phase of each cycle.

Cycling conditions for Bio-Rad CFX96 and Qiagen Rotor-Gene Q:

Phase	Description	No. of Cycles	Temperature	Duration
1	*Taq* activation	1	95 °C	7 min
2	Amplification	42	95 °C	12 sec
			*64 °C	20 sec

**Data acquisition at annealing phase*

Cycling conditions for ABI 7500 Fast:

ABI 7500 Fast Settings	
Ramp speed	Standard
Passive reference	None

Phase	Description	No. of Cycles	Temperature	Duration
1	*Taq* activation	1	95 °C	7 min
2	Amplification	42	95 °C	12 sec
			*64 °C	25 sec

**Data acquisition at annealing phase*

8. Performance Characteristics

8.1. Analytical Sensitivity

Analytical sensitivity (limit of detection) is defined as the lowest concentration at which the assay can detect with a positivity rate of at least 95%. analyzing a dilution series of a quantitated MTB H37Ra (ATCC 25177) DNA. The reactions were performed using the *ab*TES™ MTB qPCR I Kit in 5-replicates on 4 different days from 0.0025 to 1 x 10^5 CFU/ul (total of 20 replicates per dilution). The analytical sensitivity was estimated by probit analysis using SPSS release 16.0.0.

The analytical sensitivity (not in consideration of extraction) was determined to be 0.028 CFU/ul.

8.2. Analytical Specificity

The assay was tested for potential cross-reactivity against the following panel of 32 organisms. No cross-reactivity was observed.

Organisms Tested for Analytical Specificity	
Bordetella pertussis	*Mycobacterium celatum*
Chlamydophila pneumoniae	*Mycobacterium kumamotonense*
Chlamydia trachomatis	*Mycobacterium gordonae*
Corynebacterium diphtheriae	*Mycobacterium intracellulare*
Coxsackie a6	*Mycobacterium marinum*
Coxsackie b5	*Mycobacterium smegmatis*
Cytomegalovirus	*Mycobacterium avium* sub sp. Paratuberculosis
Echovirus 5	*Staphylococcus aureus*
Enterovirus 71	*Staphylococcus epidermidis*
Escherichia coli (vtec)	*Streptococcus pneumoniae*
Epstein-barr virus	*Streptococcus pyogenes*
Human Adenovirus 1	*Streptococcus salivarius*
Human respiratory syncytial	Varicella zoster virus
Mumps	Influenza A Pandemic H1N1/2009
Mycobacterium abscessus	Influenza A H3N2
Mycobacterium avium	Influenza B Virus

8.3. Precision/Reproducibility

The inter-assay and intra-assay precision was determined by performing the assay once per day in 5-replicates over a period of 4 days for 4 samples with different concentrations (total of 80 reactions per target).

For all the 4 targets, the qualitative results of all 80 reactions were 100% reproducible.

The coefficient of variation (CV) of the cycle threshold (Ct) for the intra- and inter-assay precision are as follows:

	10^5 CFU/µl	10^3 CFU/µl	10 CFU/µl	0.1 CFU/µl
Inter-assay	2.5%	2.6%	1.5%	1.0%
Intra-assay	1.1%	0.7%	0.5%	0.9%

8.4. Diagnostic Evaluation

A total of 112 clinical samples comprising of 32 culture positive and 80 culture negative samples were used for diagnostic evaluation by comparing the results with culture as the reference method.

The clinical sensitivity and specificity are as follows:

Reference Method	*ab*TES (*n*=112)		Sensitivity/ Specificity
	MTB Positive	MTB Negative	
MTB Positive	26	6	81.3% sensitivity
MTB Negative	1	79	98.8% specificity
Total	27	85	

AITbiotech Pte Ltd
25 Pandan Crescent #05-15, TIC TECH Centre, Singapore 128477
(65) 6778 6822 • sales@aitbiotech.com • www.aitbiotech.com

9. Interpretation of Data

A sample will be considered as having a positive result if the fluorescence level is higher than the threshold value and will be considered negative, otherwise.

As the kit contains an internal control, all specimens that are negative for MTB (FAM) should be positive at the internal control channel (Texas Red). A negative internal control in this case may indicate a presence of PCR inhibitors in the sample, a problem during the extraction step or a problem with the PCR reaction. The internal control may not necessarily be positive if the sample is positive for MTB due to competition of reagents.

Result	MTB (FAM)	Internal Control (Texas Red)
Negative	-	+
Positive	+	+ or -
Indeterminate	-	-

10. Troubleshooting

10.1. No signal observed with positive control and/or negative control

- Check programmed temperature settings against the protocol given.
- Affirm if proper storage was done and check the expiry date on the kit; use a newer kit to repeat experiments if needed.
- Check proper volume of the reagent added during the PCR setup.

10.2. Signal detected for negative control

- A contamination in the reagents is highly possible.
- Repeat experiment protocol and take steps to locate source of contamination.

10.3. Weak or no signal of the internal control and no sign detection in analytical channel as well

- A possible PCR inhibition has occurred. Re-extract the sample to remove inhibitors and repeat PCR, if needed.
- Affirm if proper storage was done and check the expiry date on the kit. Use a new kit to repeat the test if needed.

11. Explanation of Symbols

IVD	*In vitro* diagnostic medical device
REF	Catalogue number
-15°C -25°C	Store at -25°C to -15°C
	Manufacturer
EC REP	Authorized representative in the European community
LOT	Lot number
	Use by
Σ	Contains sufficient for <n> tests

Electronic copy of the product insert can be downloaded at http://aitbiotech.com/mtb/

AITbiotech Pte Ltd
25 Pandan Crescent #05-15, TIC TECH Centre, Singapore 128477
(65) 6778 6822 • sales@aitbiotech.com • www.aitbiotech.com