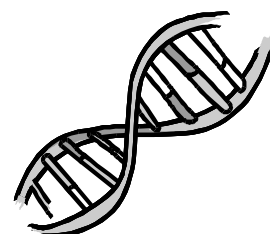



MutaPLATE[®] Mycobacterium tuberculosis *real time* PCR Kit



Qualitative assay for the specific detection of Mycobacterium tuberculosis (complex) using open (e. g. microtiterplate) *real time* PCR systems (e. g. Applied Biosystems or Stratagene).

REF KV1935196 



For in vitro diagnostic use only



1. INTENDED USE

The **MutaPLATE® Mycobacterium tuberculosis** *real time* PCR kit is a qualitative screening assay for specific detection of *Mycobacterium tuberculosis* (complex) in clinical specimens (gut biopsies, stool samples) using open *real time* PCR systems (e. g. Applied Biosystems, Stratagene).

2. INTRODUCTION

Tuberculosis is – along with AIDS and Malaria – the most widely spread infectious disease worldwide. The World Health Organization (WHO) estimated that a third of the world's population is infected and tuberculosis accounts for three million deaths annually. One-fifth of all deaths in adults in developing countries relate to TB. Two-thirds of the world's tuberculosis-infected people reside in Asia and this will have a significant impact on the control of TB in other countries as a result of increased immigration.

TB is caused by the tubercle bacillus *Mycobacterium tuberculosis* and rarely by *M. bovis* or *M. africanum*. The initial pulmonary infection usually goes unnoticed with lesions healing, sometimes leaving traces of calcified scar tissue. The infection may however progress to pulmonary tuberculosis, or through blood or lymphatic spread produce military, meningeal or other extrapulmonary involvement.

The key to interrupting the chain of transmission is the early diagnosis and treatment of pulmonary TB. For many years, diagnosis was based on staining smears for acid-fast bacilli (AFB) and culturing for mycobacteria. More recently, a number of nucleic acid amplification methods have been developed and made commercially available for rapid detection and identification of *Mycobacterium tuberculosis* complex (MTB) in clinical specimens.

3. PRINCIPLE OF THE TEST

The **MutaPLATE® Mycobacterium tuberculosis** *real time* PCR kit contains specific primers, hybridisation probes and additional material for the detection of all bacteria from *Mycobacterium tuberculosis* complex. Starting material for DNA extraction are clinical samples from the respiratory or bronchial tract (e. g. sputum, lung biopsy).

The amplification of a gene fragment specific for *Mycobacterium tuberculosis* during PCR (polymerase chain reaction) process is done by the use of thermostable DNA polymerase.

In the same step the specificity of the generated amplicon is proofed by hybridisation with probes (with fluorophor and quencher labelled oligonucleotides) specific for *Mycobacterium tuberculosis* (*real time* PCR).

In case of *Mycobacterium tuberculosis* specific amplification, the emitted fluorescence signal is detected and quantified by the *real time* PCR microplate system's optical unit (ABI: PRISM SDS-, Stratagene: MxPRO-Software).

Mycobacterium tuberculosis specific amplification is measured by **FAM** fluorescence (470 nm excision/ 510 nm detection).

To exclude a possible PCR inhibition, the amplification mix contains an internal control. The amplification of this internal control does not affect the *Mycobacterium tuberculosis* detection and is measured by a probe's **VIC / HEX** fluorescence (530 nm excision/ 555 nm detection).

4. KIT CONTENT

Each kit contains enough reagents to perform 96 tests. Each kit also contains a package insert.

Reference	Type of reagent	Presentation	Cap color
A1	amplification Mix (ready to use)	3 x 1,4 ml	blau
A2	positive control	1 x 0,2 ml	rot
A3	negative control	1 x 0,2 ml	grün

5. TEST PERFORMANCE

Required materials - provided:

- Reagents for the real time PCR in open system (e. g. microtiter plate formate)
- Package insert

Required materials - not provided:

- Open *real time* PCR system, e. g. Applied Biosystems or a comparable instrument (e.g. from Stratagene)
- TC II - PCR reaction plate, 96 wells (Applied Biosystems) or comparable microtiter plates (respectively reaction tubes to be used for optical detection within the two-parts holding frame, Applied Biosystems)
- Optical adhesive covers for microtiter plates
- DNA extraction kit for clinical samples
- Pipets (0.5 µl – 200 µl)
- sterile filter tips

6. STORAGE AND HANDLING

- All reagents (A1 to A3) should be stored at -20°C.
- Do not freeze and thaw the reagents A1, A2 several times. If used sporadically, prepare suited aliquots of the reagents and freeze again immediately.
- Cool all reagents during the working steps.
- Protect amplification mix (A1) from light (storage in the dark).
- All reagents can be used until the expiration date printed on the labels.

7. WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- This assay needs to be carried out by skilled personnel.
- Clinical samples should be regarded as potentially infectious materials.
- This assay needs to be run according to GLP (Good Laboratory Practice).
- Do not use the kit after its expiration date.

AMPLIFICATION

The PCR technology is utmost sensitive. Thus, amplification of a single molecule generates millions of identical copies. These copies may evade through aerosols and sit on surfaces. In order to avoid contamination of samples with DNA which previously was amplified, it is important to physically strictly divide sample and reagent preparation units from sample amplification units. Set up two separate working areas:

- 1) Isolation of the DNA
- 2) Amplification/ detection of amplification products

Pipets, vials and other working materials should not circulate among working units!

- Use always sterile pipette tips with filters
- Wear separate coats and gloves in each area
- Routinely decontaminate your pipettes and the laboratory benches with decontaminant
- Avoid aerosols

8. PROCEDURE

The complete procedure is separated in three steps:

- A) DNA extraction
- B) Amplification and combined detection of DNA fragments using the specific probes.
- C) Interpretation of the results using the ABI: PRISM SDS- (respectively the Stratagene: MxPRO-) software.

A) DNA-EXTRACTION

- 1) Extract bacterial DNA from clinical respiratory/ bronchial samples by use of a commercial DNA isolation kit (suited for throat swabs, sputum etc.) according to the manufacturer's instructions.
- 2) If (TaqMan) PCR is not performed immediately, store the extracted DNA at -20°C .

B) *Real time* Mycobacterium tuberculosis (MutaPLATE[®]) PCR-PROTOCOL

Please carefully read the manufacturer's instructions before starting the procedure!
Each assay should include a negative and positive control. Use filter tips for all pipetting:

- 1) Pipet **40 μl** of the *ready to use* amplification mix (A1) per well of a 96 wells optical microtiter plate. The number of wells used is calculated from the number of samples plus one positive and one negative control.
- 2) Add **10 μl** of sample DNA or positive or negative control (A2 and A3) per well, respectively. Mix by up and down pipetting. Pipet the negative control first. To avoid contamination, it is advisable to cover the wells containing the negative control with an adhesive seal while pipetting the positive control and sample DNA. Remove this adhesive seal after preparing all wells.

Cover the 96 wells optical microtiter plate with optical adhesive cover and transfer to the (open) *real time* PCR system.

- 3) Run the PCR using following temperature protocol:

95°C for **10 min**

40 cycles of:

95°C for **20 sec**

55°C for **60 sec**

72°C for **20 sec**

measurement at end of this step

measurement at end of this step

(for ABI7000 instrument choose here **30 sec** !)
(fluorescence emission data of both steps are combined)

C) PCR ANALYSIS AND INTERPRETATION OF RESULTS

Mycobacterium tuberculosis specific amplification is detected by FAM fluorescence (at 510 nm). The internal control is measured by VIC / HEX fluorescence (at 555 nm).

Use following settings to define a reporter and quencher with the ABI PRISM SDS (or comparable) software:

Detection	Reporter	Quencher
<i>Mycobacterium tuberculosis</i> DNA	FAM	none
Internal Control	VIC / HEX	none

Following results can arise:

- 1) FAM fluorescence is detected.
The result is positive: The sample contains *Mycobacterium tuberculosis*.
 The occurrence of VIC / HEX fluorescence is inessential as high concentrations of *Mycobacterium tuberculosis* DNA may reduce or even inhibit the amplification of the internal control.
- 2) No FAM, but VIC / HEX fluorescence is detected.
The result is negative: The sample does not contain *Mycobacterium tuberculosis*. The detected signal of the internal control excludes the possibility of an inhibition of the PCR.
- 3) Neither FAM, nor VIC / HEX fluorescence is detected.
A diagnostic statement can not be made.
 An inhibition of the PCR reaction occurred.

