

GeneProof®

Mycobacterium tuberculosis

PCR Kit



in vitro Diagnostics

The kit is designed for professional use in specialized clinical and research laboratories.

Method principles

This kit is designed for the detection of the *Mycobacterium tuberculosis* genomic DNA based on the detection of the specific multi-copy insertion sequence ITS6110, using the “one tube nested” method of the Polymerase Chain Reaction (PCR) and the detection of the resulting amplification products by agarose gel electrophoresis. This kit provides for specific detection of species belonging into the *Mycobacterium tuberculosis* complex group (*M. tuberculosis*, *M. bovis*, *M. africanum*, *M. microti* and BCG). The detection kit takes an advantage of the “hot start” technology, minimizing non-specific reactions and assuring maximum sensitivity. The reaction mix includes an internal standard controlling the possible inhibition of the PCR reaction and the uracil-DNA-glycosylase (UDG) controlling possible contamination of the PCR reaction by amplification products. This kit is designed for qualitative detection.

Kit composition

	Cat. No. MT/E/ISIN/025 25 reactions	Cat. No. MT/E/ISIN/050 50 reactions	Cat. No. MT/E/ISIN/100 100 reactions
MasterMix			
<i>Mycobacterium tuberculosis</i>	1 x 900 µl	2 x 900 µl	4 x 900 µl
Positive control			
<i>Mycobacterium tuberculosis</i> 10 ² copies/µl	1 x 50 µl	1 x 50 µl	2 x 50 µl

Storage and transportation conditions

Transport the kits at temperatures ranging from -20 °C to -80 °C. The kit remains stable for at least 6 months from the date of manufacturing at the temperature of -20°C. Repeated freezing and thawing of the MasterMix and the Positive control may result in lower detection quality. The manufacturer therefore recommends to aliquot the MasterMix by 36 µl directly to PCR tubes and hold in stock at -20°C. Positive control may be held in stock at 4°C.

User Manual

Sampling and sample storage

Samples are usually taken from the **respiratory tract** for the purpose of *M. tuberculosis* detection. Infection is usually not disseminated; therefore a detection in peripheral blood samples is usually not valuable for the diagnosis purposes. Samples of **sputum**, **bronchoalveolar lavages**, **bronchial and tracheal aspirates**, or possibly of **lung biopsies** (about 1 g), **urine** (clean-catch midstream urine, morning urine), **skin ulceration**, **pus**, **abscess contents** or **synovial fluids** must be sampled in a sterile way and inserted into a tube without any transportation media. Store and transport at the temperature of 4 °C within 48 hours. If longer storage period is required, freeze to -20 to -80 °C.

DNA isolation

Isolation recommended by means of commercial DNA isolation kits according to the particular protocols of the isolation kit manufacturers. The manufacturer recommends the following isolation kits: PathogenFree DNA isolation kit (GeneProof); QIAamp DNA Blood Mini Kit (QIAGEN); NucleoSpin Blood (Macherey-Nagel).

PCR amplification

1. Add **36 µl of the MasterMix** and **4 µl of the DNA isolate** or **4 µl of the Positive control** into the PCR tube. The final reaction mix volume should be **40 µl**.*
2. Insert the tubes into a thermocycler and amplify them according to the following recommended program:

Amplification program: **

UDG decontamination	37 °C/2 min.
initial denaturation	96 °C/10 min.
denaturation	96 °C/20 sec.
annealing	70 °C/20 sec.
extension	72 °C/40 sec.
number of cycles	30
denaturation	96 °C/20 sec.
annealing	60 °C/20 sec.
extension	72 °C/30 sec.
number of cycles	40
final extension	72 °C/2 min.

*Tubes should be maintained in cold environment when handled (at 0 – 15 °C)

**Hereby presented amplification conditions may differ for various types of thermocyclers. The above listed conditions apply for PTC 200 (MJ Research) thermocycler.

Agarose electrophoresis

The resulting amplification products should be separated on 2% agarose gel electrophoresis (5V/cm) containing Ethidium bromide (5 µg/1 ml) and visualized by means of UV illumination. Since the loading buffer is included in the reaction mix, there should be directly loaded at least 10 µl of the amplification product.

Detection evaluation

Positive sample – is the detected amplification product with the length of 197 bp, there may also be the detected amplification product of the internal standard with the length of 460 bp (Fig. 1).

Negative sample – only the amplification product of the internal standard with the length of 460 bp is detected.

Inhibiting sample - should inhibition of the reaction components occur or should the reaction fail to proceed, none of the above-listed amplification products would be detected and the examination should be repeated.

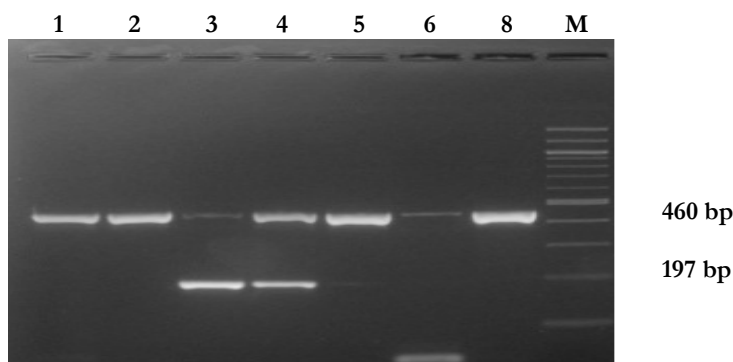


Fig. 1. Electrophoretical detection of amplification products on 2% agarose gel. 197 bp long fragment results from the amplification of the target sequence of ITS6110 *Mycobacterium tuberculosis*, 460 bp long fragment results from the amplification of the internal standard. **Tracks No. 1, 2, 5 and 8** – results of the negative detection, **tracks No. 3 and 4** – results of the positive detection *Mycobacterium tuberculosis* in the sample, **track No. 6** – partial inhibition of the sample, **M** - 100 bp weight marker.

Warning:

- The kit is manufactured according to the European IVD Directive 98/79/EC.
- Be very careful when handling the Positive control or the clinical material – incorrect handling could result in contamination and the consequent impairment of the kit components or the MasterMix! The manufacturer is not responsible for the kit impairment due to incorrect handling.
- The kit should be disposed of after use according to the current legal regulations considering the fact that the kit doesn't contain any dangerous, infectious or toxic components that would be subject to special safety regulations and the packaging materials are made of paper and polypropylene.
- Service fee assuring returnability and recycling of the waste packaging material has been paid under the identification number EK-F00041495.

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