

GeneProof®

Mycobacterium tuberculosis

PCR Kit



in vitro Diagnostics

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

Kit composition

Cat. No.	Internal Standard is included in the MasterMix for inhibition control			Contains a separate tube of Internal Standard for inhibition and isolation process control		
	MT/ISIN/025 25 reactions	MT/ISIN/050 50 reactions	MT/ISIN/100 100 reactions	MT/ISEX/025 25 reactions	MT/ISEX/050 50 reactions	MT/ISEX/100 100 reactions
MASTERMIX <i>Mycobacterium tuberculosis</i>	1 x 750 µl	2 x 750 µl	4 x 750 µl	1 x 750 µl	2 x 750 µl	4 x 750 µl
POSITIVE CONTROL <i>Mycobacterium tuberculosis</i> 10 ² copies/µl	1 x 200 µl	1 x 200 µl	2 x 200 µl	1 x 200 µl	1 x 200 µl	2 x 200 µl
INTERNAL STANDARD <i>Mycobacterium tuberculosis</i>	-	-	-	1 x 1000 µl	1 x 1000 µl	2 x 1000 µl

Storage and transportation conditions

Transport the kits at temperatures ranging from -20°C to -80°C. The kit remains stable for 9 months from the date of manufacturing at the temperature of -20°C. Repeated freezing and thawing of the MasterMix, Internal Standard or the Positive control may result in lower detection quality. The manufacturer therefore recommends to aliquot the MasterMix by 30 µl directly to PCR tubes and hold in stock at -20°C. Positive control and the Internal Standard may be held in stock at 4°C.

Pathogen information

Mycobacterium tuberculosis is a causal agent of a serious respiratory disease – tuberculosis. Significance of this disease currently grows due to the occurrence of new multi-resistant strains, due to the growing numbers of immunodeficient patients and due to the growing population migration rates. The infection is located mostly in lungs and mycobacteria can be demonstrated in sputum, BAL and lungs biopsy samples; there also appear abnormal infection locations (basilar meningitis, gangliform tuberculosis, urogenital infections, joint infections, eye infections). You can also see rare post-vaccination complications after the application of the BCG vaccine. Classic tests designated for tuberculosis diagnostics are based on the acidophilic coloring of mycobacteria and microscopy accompanied by cultivation confirmation. These traditional methods are rather time consuming (cultivation requires 2-4 weeks) and their sensitivity is low. Therefore the sensitive and quick PCR methods gradually become standard procedures in the tuberculosis diagnostics – these methods are able to register even tiny numbers of mycobacteria in any clinical material. *Mycobacterium tuberculosis* is a customary pathogen and positivity of a PCR examination must be considered a proof of the tuberculosis disease. PCR method detection could be negative in case of extra-pulmonary localization or latent carrier state and due to inadequate clinical sampling. There could also appear problems in the interpretation of long-term, repeatedly positive detections (in any types of samples) in senior, asymptomatic, “latently infected” patients, where mycobacteria may be present in very low amounts and don’t react to any treatment. Possible, several-weeks lasting persistence of dead mycobacteria must be taken into account when interpreting PCR results after an anti-TBC therapy.

Method principles

The kit is designed for the detection of the multi-copy insertion sequence ITS6110 DNA *Mycobacterium tuberculosis* by means of the Polymerase Chain Reaction (PCR) and for measuring of the amplification product concentration growth in the course of the PCR by means of the fluorescence marked probe (real-time PCR). This method specifically detects strains of the *Mycobacterium tuberculosis* complex (*M. tuberculosis*, *M. bovis*, *M. africanum* and *M. microti*); it also detects vaccination strains (e.g. BCG). *M. tuberculosis* presence is indicated by FAM fluorophore fluorescence growth. An Internal Standard (IS) is included in the reaction mix, controlling the possible inhibition of the PCR reaction or the efficiency of the DNA isolation process. IS positive amplification is detected in the fluorescence channel for the JOE fluorophore. The detection kit takes advantage of the “hot start” technology, minimizing non-specific reactions and assuring maximum sensitivity. It contains uracil-DNA-glycosylase (UDG), eliminating possible contamination of the PCR reaction by amplification products. Sensitivity of the PCR detection kit runs in single copies of the IS6110 insertion sequence in a reaction. Multiple copy of these sequences in mycobacterial genome providing very high sensitivity of laboratory diagnostics of body fluids (bronchoalveolar lavage, urine) or sputa. The sensitivity is 16 times higher in comparison to the single gene detection. The kit is designed for *in vitro* diagnostics and provides qualitative detection.

GeneProof PCR kits are designed for use with real-time devices from various manufacturers.

Mycobacterium tuberculosis PCR Kit has been validated with the following devices:

Rotor-Gene™ 3000 (Corbett Life Science)
Rotor-Gene™ 6000 (Corbett Life Science)
7500 Real-Time PCR System (Applied Biosystems)
LightCycler® 2.0 (Roche)
LightCycler® 480 System (Roche)
SLAN Real-time Quantitative PCR Fluorescent Detection System (Shanghai Odin Scienc & Technology Co.)

For detailed information about PCR kit use with specific devices see the Manufacturer’s web site (www.geneproof.com) or request the information from your kit supplier.

If you want to use the kit with other real-time devices, contact the manufacturer, please: support@geneproof.com

Warning:

- The kit has been manufactured according to the EC Directive 98/79/EC as an *in vitro* medical diagnostic device.
- 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.

User Manual

Sampling and sample storage

Samples are usually taken from the respiratory tract for the purpose of *M. tuberculosis* demonstration. Infection is usually not disseminated; therefore a demonstration 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

DNA isolation should be performed by isolation kits available at the market according to specific protocols for the particular microorganism isolation. The manufacturer recommends the following isolation kits: PathogenFree DNA Isolation Kit (GeneProof).

All GeneProof PCR kits include an Internal Standard (IS) providing for an effective monitoring of eventual inhibition of the PCR amplification and also of the isolation process efficiency. The Internal Standard is a precisely defined and quantified construct of a plasmid and insert, prepared by genetic engineering methods GeneProof develops and sells two basic variants of PCR kits which differ in the Internal Standard composition.

PCR Kit ISIN (Cat. No. MT/ISIN...)

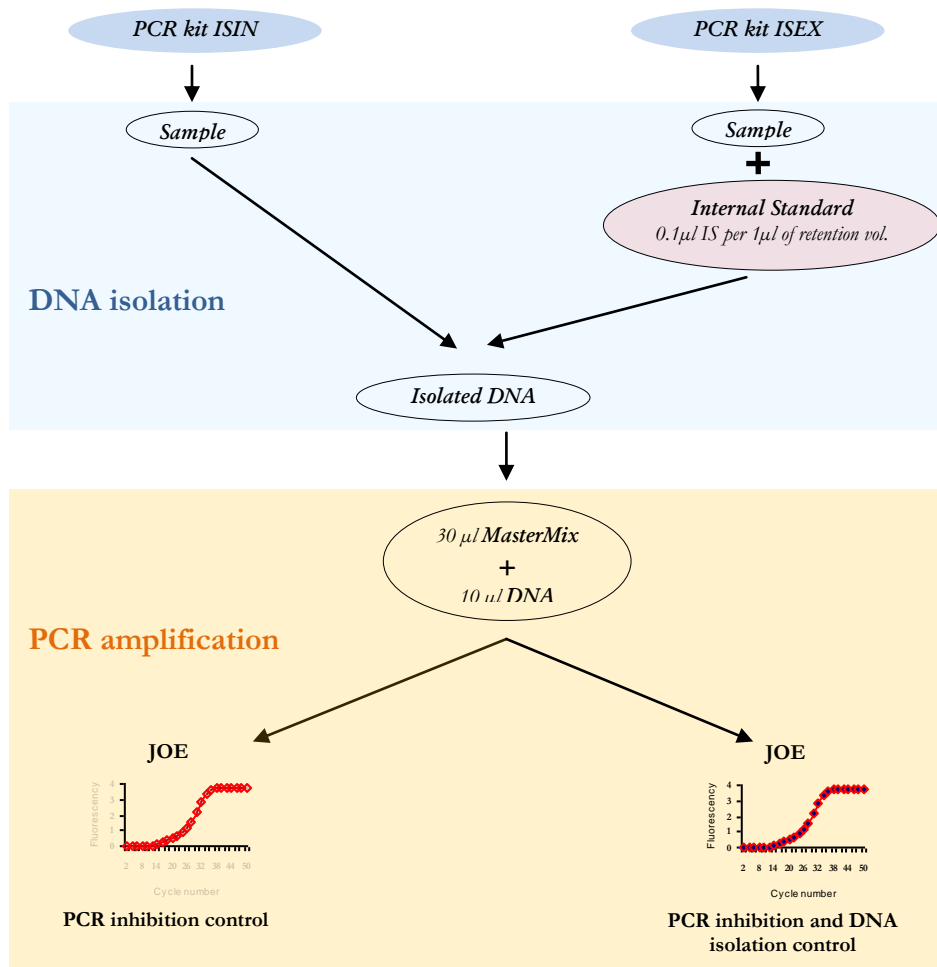
In this version of the PCR kit the Internal Standard (IS) is included directly in the MasterMix tube. This version of the kit provides **efficient control of the PCR reaction inhibition**.

PCR Kit ISEX (Cat. No. MT/ISEX...)

In this PCR kit version the Internal Standard (IS) is included in a separate tube within the package. This version of the PCR kit can be used for both **PCR reaction inhibition control** and **DNA isolation efficiency control**.

When using the ISEX versions of the PCR kits the IS should be added directly into the sample at the beginning of the isolation process so that in the end 1 µl of the resulting elution volume contains 0.1 µl of the IS:

Elution Volume	25 µl	50 µl	100 µl	200 µl
Internal Standard	2.5 µl	5 µl	10 µl	20 µl



PCR amplification

1. Add **30 µl of the MasterMix** and **10 µl of the DNA isolate** or **10 µl of the Positive Control** into a PCR tube. The final reaction mix volume should be 40 µl.
2. Close the tubes, centrifuge shortly, insert into the device and program according to the following table:

Amplification program:

UDG decontamination	37°C/2 min.
initial denaturation	95°C/10 min.
denaturation	95 °C/5 sec.
annealing	60°C/40 sec. - reading of the fluorescence signal
extension	72°C/20 sec.
number of cycles	45

Qualitative evaluation of detection

