

T-Track® TB

RT-qPCR assay as an aid in the diagnosis of *M. tuberculosis* infection (including active disease), via highly sensitive determination of the relative expression of TB antigen-induced, specific markers.

According to the World Health Organization (WHO), tuberculosis (TB) is one of the top 10 causes of death worldwide. TB is caused by *Mycobacterium tuberculosis*. It typically affects the lung (pulmonary TB) but can also affect other organs (extrapulmonary TB). About 25% of the world's population is latently infected with *M. tuberculosis* and at risk of developing active TB disease if not treated. Patients under immunosuppression, e.g. therapeutically induced in case of transplantations or rheumatic disorders, are especially at risk. T-Track® TB is a qualitative diagnostic test, suitable for screenings, to determine TB-specific reactivity of T-cells. Based on a patented technology, this RT-qPCR-based test is intended for use as an aid in the diagnosis of *M. tuberculosis* infection (including active disease). The T-Track® TB assay consists of two diagnostic kits:

- 1. T-Track® TB Stimulation:** Stimulation of TB-specific immune cells, via incubation with TB antigens, followed by stabilization of RNA.
- 2. T-Track® TB Quant PCR:** Measurement of the relative expression of specific classification markers via reverse transcription and quantitative PCR (RT-qPCR). Software based analysis.

Differential expression levels of the classification markers in stimulated and unstimulated blood samples may indicate an infection with *M. tuberculosis*. Results must be interpreted within the context of all relevant clinical and laboratory findings.

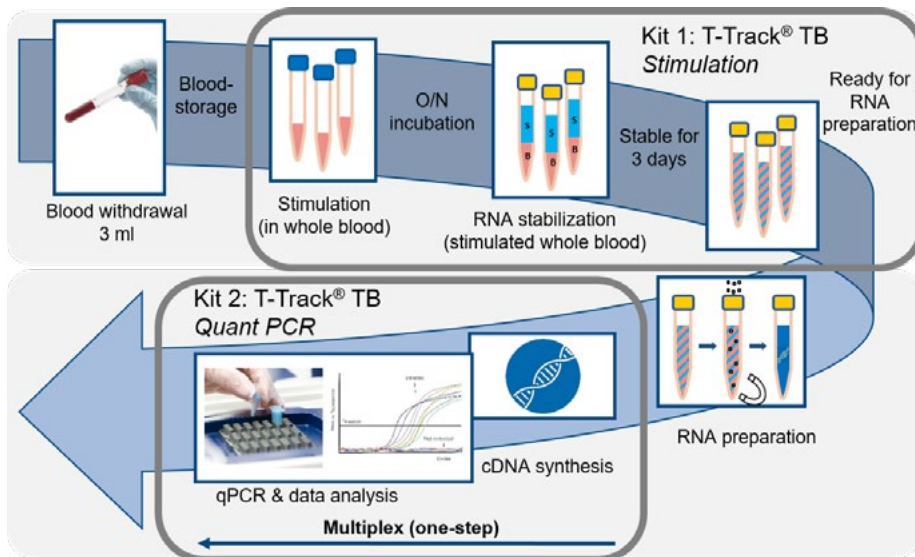


T-Track® TB : T-cell assay for improved diagnostics

- **CE-marked IVD (in vitro diagnostic product) – RT-qPCR**
- Aid in the diagnosis of *M. tuberculosis* infection (including active disease)
- Highly-sensitive, patented technology: Including marker also for the early phase of infection
- Measurement and analysis of the relative expression of the specific markers IFNG and CXCL10, after *in vitro* stimulation of immune cells with the TB antigens ESAT-6 und CFP-10
- Easy handling, standardized and semi-automated processing
- Software based analysis

T-Track® TB	Kit 1: T-Track® TB Stimulation	Kit 2: T-Track® TB Quant PCR
Kit components	<ul style="list-style-type: none"> • TB antigens ESAT-6 and CFP-10 • Stimulation Control (PHA) • RNA Stabilizer • Instructions for use 	<ul style="list-style-type: none"> • Reaction Mix (DNA polymerase) • Enzyme (reverse transcriptase) • Primer & Probe-Mix • Positive Control • Negative Extraction Control • Instructions for use • T-Track® TB Analysis Tool software
TB antigens/marker	<p>ESAT-6: early secretory antigenic target (6 kDa)</p> <p>CFP-10: culture filtrate protein (10-kDa)</p>	<p>IFNG: Interferon gamma</p> <p>CXCL10 (IP-10): C-X-C motif chemokine 10</p>
Stimulation/RT-qPCR	The stimulation of human whole blood with TB antigens leads to reactivation of TB-specific immune cells and induces the expression of specific markers.	Measurement of expression levels of TB-specific expressed mRNA. Standardization via RPLP0 (house keeping gene) and analysis using a software tool.
Starting material	3 ml whole blood (heparinized)	Stabilized RNA from kit 1

Test principle and procedure



- **Blood withdrawal:** Heparinized blood needed (only 3 ml per patient)
- **Stimulation:** Stimulation via *M. tuberculosis* proteins ESAT-6 & CFP-10; no cell preparation necessary
- **RNA stabilization:** Ready to use stabilizer; sample storage possible after stabilization (up to 72 hours)
- **RNA preparation**
- **RT-qPCR:** Multiplex, incl. internal control
- **Easy evaluation:** T-Track® TB Analysis Tool software

Evaluation of clinical sensitivity and specificity

The suitability of T-Track® TB as an aid in the diagnosis of TB infection was evaluated in 107 donors in total. 51 of 53 patients suffering from an active TB disease (culture-confirmed or verified by the combination of PCR and microscopy results) were classified as "positive" via T-Track® TB. This corresponds to a sensitivity of 94.8%. In a second group of patients who have no diagnosis for TB infection, 49 of 54 patients were classified as "negative" via T-Track® TB. This corresponds to a specificity of 94.0%.

	Sensitivity	Specificity	Precision
T-Track® TB	94.81% (CI 87-98.36)	94.03% (CI 85.2-98.09)	94.44% (CI 89.26-97.32)

In 1.4% of the cases no evaluation could be performed. CI, confidence interval

Evaluation of the results with T-Track® TB Analysis Tool software

MIKROGEN DIAGNOSTIK

T-Track® TB Analysis Tool 1) IVD

Version v01.01_TB_EN
Date 10.12.2021

T-Track® TB result **positive**

Sample ID: ID1234
Operator:
TB Stim. Lot¹⁾: TTB
TB Quant PCR Lot¹⁾: TQP

Comment:
User input

Unstimulated	Ct input	Input check
CXCL10 Ct	35.6	
IFNG Ct	29.8	
RPLP0 Ct	25.1	

TB Antigen Stimulated	Ct input	Input check	Fold Changes
CXCL10 Ct	26.8		CXCL10 FC 228.04
IFNG Ct	27.3		IFNG FC 4.96
RPLP0 Ct	24.8		

Stimulation Control (PHA)	Ct input	Input check	Fold Changes
CXCL10 Ct	25.5		CXCL10 FC 411.34
IFNG Ct	25.8		IFNG FC 10.02
RPLP0 Ct	24.3		

Negative Control (pNC)	Ct input	Input check
CXCL10 Ct	Undetermined	
IFNG Ct	Undetermined	
RPLP0 Ct	Undetermined	

Positive Control (PC)	Ct input	Input check
CXCL10 Ct	29.8	
IFNG Ct	29.0	
RPLP0 Ct	29.4	

¹⁾ "T-Track® TB Analysis Tool" is only to be used in combination with "T-Track® TB Stimulation" and "T-Track® TB Quant PCR"

Quality Control	
0 Prerequisite for threshold correction	fulfilled
1 Negative Extraction Control (pNC)	valid
2 Positive Control (PC)	valid
3 RPLP0 Internal control	valid
4 RPLP0 Ct of Stimulation control (PHA)	valid
5 lower measuring range	valid
6 Stimulation control (PHA)	valid
7 Sample assignment	valid
8 CXCL10 single marker analysis	n.d.
9 IFNG single marker analysis	n.d.
10 Classification markers	valid
Overall QC result	valid

The T-Track® TB Analysis Tool is a software for qualitative evaluation of measured values determined by T-Track® TB.

The Ct-values measured for each patient sample and controls by RT-qPCR can be entered in the T-Track® TB Analysis Tool. The software checks sample validity and calculates the qualitative classification results.

Positive:

TB-reactive T-cells were detected. An infection with *M. tuberculosis* (latent/active) is probable.

Negative:

TB-reactive T-cells were not detected. An infection with *M. tuberculosis* (latent/active) is not probable.

Minimal system requirements:

- Microsoft® Windows® 7 with service pack 1 (SP1)
- .Net Framework Version 4.5
- Excel 2013 or higher

Article no.

11001004 **T-Track® TB Stimulation** (Reagents for the analysis of 10 patient samples)
11001005 **T-Track® TB Quant PCR** (34 reactions)

Storage

at +2°C - +8°C
at -25°C - 15°C