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IVD

For professional use only

M.tuberculosis - M.bovis
REAL-TIME PCR Detection Kit
INSTRUCTION FOR USE

"DNA-Technology Research & Production", LLC,

142281, Russia,

Moscow Region, Protvino,

Zheleznodorozhnaya Street, 20

Phone/fax: +7(495) 640.17.71

E-mail: info@dna-technology.com

https://www.dna-technology.com

Customer service department

E-mail: hotline@dna-technology.ru

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R1-P404-23/4EU

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1. INTENDED USE

The **M.tuberculosis - M.bovis REAL-TIME PCR Detection Kit** is intended for research and diagnostic applications. The **M.tuberculosis - M.bovis REAL-TIME PCR Detection Kit** is an *in vitro* Nucleic Acid Test (NAT) – pathogen-detection-based product. The **M.tuberculosis - M.bovis REAL-TIME PCR Detection Kit** is designed to detect *Mycobacterium tuberculosis* and *Mycobacterium bovis* nucleic acids in human biological samples with an aid of Polymerase Chain Reaction (PCR) method. Samples are human biological materials: cerebrospinal fluid, biopsy material or punctate from lesions of organs and tissues, bronchoalveolar lavage, phlegm, urine, ejaculate.

Indications for the use: symptoms of tuberculosis, monitoring the effectiveness of therapy, differential diagnosis of infections with similar clinical implications, complex diagnostics of patients with infertility.

The application of the kit does not depend on population and demographic aspects. There are no contradictions for use of the **M.tuberculosis - M.bovis REAL-TIME PCR Detection Kit.**

The **M.tuberculosis - M.bovis REAL-TIME PCR Detection Kit** can be used in clinical and diagnostic laboratories of medical institutions and research practice.

Potential users: personnel qualified in molecular diagnostics methods and working in the clinical and diagnostic laboratory.

It is necessary to apply the kit only as directed in this instruction for use.

2. METHOD

The implemented PCR method is based on amplification of a target DNA sequence. To increase the sensitivity and specificity of the amplification reaction, the use of a hot-start is provided. Hot-start is provided by reaction mixture preparation consisting of two layers separated by a layer of paraffin or the use of Taq-polymerase blocked by antibodies. The polymerase chain reaction starts only when paraffin is melted or thermal dissociation of a complex of Taq polymerase and antibodies is happened. It excludes non-specific annealing of primers to targets DNA in the initial heating of the tube.

The **M.tuberculosis - M.bovis REAL-TIME PCR Detection Kit** is based on fluorescent modification of the PCR method. The PCR-mix contains two target-specific probes bearing reporter fluorescent dyes (Fam and Hex) and quencher molecules. Once hybridized to a target sequence, the probes become activated. As a result of activation fluorescence increases proportionally to target sequence amplification. The intensity of fluorescence is measured at every cycle of reaction with a Real-time PCR thermal cycler data collection unit and analyzed with the software provided.

The PCR-mix includes the Internal control (IC), which is intended to assess the quality of the polymerase chain reaction. DNA probe used for the detection of the *Mycobacterium* complex (*M.tuberculosis/M.bovis)* product amplification includes fluorescent dye Fam. DNA probe used for the detection of the internal control amplification product includes the fluorescent dye Hex. Table 1 shows the detection channels of amplification products.

Table 1. Detection channels of amplification products

Fam/Green	Hex/Yellow	Rox/Orange	Cy5/Red	Cy5.5/Crimson
Mycobacterium complex *(M.tuberculosis/M.bovis)*	IC	-	-	-

The automatic analysis is available on “DNA-Technology” made instruments: DTlite or DTprime REAL-TIME Thermal Cyclers for **M.tuberculosis - M.bovis REAL-TIME PCR Detection Kit** (see the catalogue at https://www.dna-technology.com to see available supply options). The current version of the software is available for download at https://www.dna-technology.com/software.

The **M.tuberculosis - M.bovis REAL-TIME PCR Detection Kit** is also approved for use with iQ (Bio-Rad Laboratories) and Rotor-Gene Q (Qiagen) real-time thermal cyclers.

3. CONTENT

The **M.tuberculosis - M.bovis REAL-TIME PCR Detection Kit** contains PCR-mix, Taq-polymerase solution, mineral oil and positive control sample. The detailed description of content is represented in Table 2.

Table 2. The **M.tuberculosis - M.bovis REAL-TIME PCR Detection Kit** content, package S (standard) for R1-P404-S3/4EU and R1-P404-23/4EU

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless transparent liquid under waxy white fraction	960 µL (20 µL per tube)	48 tubes or 6 8-tube strips
Taq-polymerase solution	Colorless transparent liquid	500 µL	1 tube
Mineral oil	Colorless transparent viscous oily liquid	1.0 mL	1 tube
Positive control	Colorless transparent liquid	130 µL	1 tube
Strip's caps [1]	6 8-caps		

All components are ready to use and do not require additional preparation for operation.
The **M.tuberculosis - M.bovis REAL-TIME PCR Detection Kit** is intended for single use and designed for 48 tests (no more than 46 defined samples, one positive control and one negative control).

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

4.1. Specimen collection

- Sterile containers to collect clinical material;
- Sterile tubes containing transport medium: "DNA-Technology" made **PREP-RAPID** (REF P-001/1EU) or **STOR-M** (REF P-910-1/1EU) or **STOR-F** (REF P-901-1/1EU, P-901-N/1EU, P-901-R/1EU) or equivalent for the transportation of the sample.

4.2. DNA extraction and PCR

Preamplification-specimen and control preparation area:

- Biological safety cabinet class II;
- Vortex mixer;
- Refrigerator;
- Nucleic acid extraction kit ("DNA-Technology" made **PREP-NA** REF P-002/1EU and **PREP-GS** REF P-003/1EU extraction kits are recommended);
- High speed centrifuge (RCF(g) no less than 16000);
- Solid-state thermostat (temperature range 50-98°C);
- Tube rack for 1.5 mL tubes;
- 1.5 mL tubes;
- Container for used pipette tips, tubes and other consumables;
- Single channel pipettes (dispensers covering 20-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 200 µL, 1000 µL);

[1] - for detection kit packaged in strips REF R1-P404-S3/4EU

– Physiological saline solution 0.9% NaCl (Sterile);
– Powder-free surgical gloves;
– Disinfectant solution.

When extracting NA from phlegm (method 1):

– 10% trisodium phosphate x $12H_2O$;
– 1M HCl solution;
– 5.0% chloramines solution;
– Distilled water.

When extracting NA from phlegm (method 2):

– Mucolysin.

Preamplification-reagent preparation area:

– UV PCR cabinet;
– Vortex mixer;
– Vortex rotor for strips (using detection kit packaged in strips REF R1-P404-S3/4EU);
– Refrigerator;
– Tube rack for 0.2 mL tubes;
– Tube rack for strips of eight 0.2 mL tubes;
– Single channel pipettes (dispensers covering 0.5-1000 µL volume range);
– RNase and DNase free filtered pipette tips (volume 20 µL, 200 µL, 1000 µL);
– Container for used pipette tips, tubes and other consumables;
– Powder-free surgical gloves;
– Disinfectant solution.

Post-Amplification – Amplification detection area:

– Real-time PCR thermal cycler.

Software:

The most recent version of the DT thermal cyclers software can be downloaded from https://www.dna-technology.com/software.

The OS supported: all versions of Windows starting from 7.

5. TRANSPORT AND STORAGE CONDITIONS

Expiry date – 12 months from the date of production.

All components of the **M.tuberculosis - M.bovis REAL-TIME PCR Detection Kit** must be stored at temperatures from 2 °C to 8 °C during the storage period. PCR-mix must be stored at temperatures from 2 °C to 8 °C and out of light during the storage period. The excessive temperature and light can be detrimental to product performance.

The kit can be transported by all types of roofed transport at temperatures from 2 °C to 8 °C over the transportation. It is allowed to transport the kit at temperatures from 2 °C to 8 °C for no more than 5 days.

Shelf-life of the kit following the first opening of the primary container:

- components of the kit should be stored at temperatures from 2 °C to 8 °C during the storage period;
- PCR-mix for amplification should be stored at temperatures from 2 °C to 8 °C and out of light during the storage period.

The kit stored in under undue regime should not be used.

An expired the **M.tuberculosis - M.bovis REAL-TIME PCR Detection Kit** should not be used.

We strongly recommend to follow the given instructions in order to obtain accurate and reliable results.

The conformity of the **M.tuberculosis - M.bovis REAL-TIME PCR Detection Kit** to the prescribed technical requirements is subject to compliance of storage, transportation and handling conditions recommended by manufacturer.

6. WARNINGS AND PRECAUTIONS

Only personnel trained in the methods of molecular diagnostics and the rules of work in the clinical and diagnostic laboratory are allowed to work with the kit.

Handle and dispose all biological samples, reagents and materials used to carry out the assay as if they were able to transmit infective agents. The samples must be exclusively employed for certain type of analysis. Samples must be handled under a laminar flow hood. Tubes containing different samples must never be opened at the same time. Pipettes used to handle samples must be exclusively employed for this specific purpose. The pipettes must be of the positive dispensation type or be used with aerosol filter tips. The tips employed must be sterile, free from the DNases and RNases, free from DNA and RNA. The reagents must be handled under a laminar flow hood. The reagents required for amplification must be prepared in such a way that they can be used in a single session. Pipettes used to handle reagents must be exclusively employed for this specific purpose. The pipettes must be of the positive dispensation type or be used with aerosol filter tips. The tips employed must be sterile, free from the DNases and RNases, free from DNA and RNA. Avoid direct contact with the biological samples reagents and materials used to carry out the assay. Wear powder-free surgical gloves. Wear protective clothing (work clothes and personal protective equipment) working with microorganisms classified as particularly pathogenic. The protective clothing and personal protective equipment must comply with the work to be performed and health and safety requirements. Avoid producing spills or aerosol. Any material being exposed to biological samples must be treated for at least 30 minutes with disinfecting solution or autoclaved for 1 hour at 121 °C before disposal.

Molecular biology procedures, such as nucleic acids extraction, PCR-amplification and detection require qualified staff to avoid the risk of erroneous results, especially due to the degradation of nucleic acids contained in the samples or sample contamination by amplification products.

All oligonucleotide components are produced by artificial synthesis technology according to internal quality control protocol and do not contain blood or products of blood processing.

Positive control is produced by artificial DNA synthesis technology. Positive control does not include parts of infectious agents.

All the liquid solutions are designed for single use and can not be used more than once in amplification reactions. Plastic tubes do not contain phthalates. Do not breathe gas/fumes/vapor/spray produced by the components of the kit. Do not eat/drink components of the kit. Avoid contact with eyes. Only use the reagents provided in the kit and those recommended by manufacturer. Do not mix reagents from different batches. Do not use reagents from third party manufacturers' kits. All laboratory equipment, including pipettes, test tube racks, laboratory glassware, lab coats, bouffant caps, etc., as well as reagents should be strictly stationary. It is not allowed to move them from one room to another. Equip separate areas for the extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never introduce an amplification product in the area designed for extraction/preparation of amplification reactions. Wear lab coats, gloves and tools, which are exclusively employed for the

extraction/preparation of the amplification reaction and for the amplification/detection of the amplification products. Never transfer lab coats, gloves and tools from the area designed for amplification/detection of the amplification products to the area designed for extraction/preparation of amplification reactions. Amplification products must be handled in such a way as to reduce dispersion into the environment as much as possible, in order to avoid the possibility of contamination. Pipettes used to handle amplification products must be exclusively employed for this specific purpose. Remove PCR waste only in a closed form. Remove waste materials (tubes, tips) only in a special closed container containing a disinfectant solution. Work surfaces, as well as rooms where NA extraction and PCR are performed, must be irradiated with bactericidal irradiators for 30 minutes before and after the work.

Do not open the tubes after amplification. Waste materials are disposed of in accordance with local and national standards. All surfaces in the laboratory (work tables, test tube racks, equipment, etc.) must be treated daily with disinfecting solution.

Emergency actions

Inhalation: Inhalation of the PCR-mix contained within this kit is unlikely, however care should be taken.

Eye Contact: If any component of this kit enters the eyes, wash eyes gently under potable running water for 15 minutes or longer, making sure that the eyelids are held open. If pain or irritation occurs, obtain medical attention.

Skin Contact: If any component of this kit contacts the skin and causes discomfort, remove any contaminated clothing. Wash affected area with plenty of soap and water. If pain or irritation occurs, obtain medical attention.

Ingestion: If any component of this kit is ingested, wash mouth out with water. If irritation or discomfort occurs, obtain medical attention.

Do not use the kit:

- When the transportation and storage conditions are breached;
- When the reagents' appearance does not respond to the kit passport;
- When the kit components packaging is breached;
- After the expiry date provided.

Significant health effects are **NOT** anticipated from routine use of this kit when adhering to the instructions listed in the current manual.

7. SAMPLES

The **M.tuberculosis - M.bovis REAL-TIME PCR Detection Kit** is designed to detect DNA extracted from cerebrospinal fluid, biopsy material or punctate from lesions of organs and tissues, bronchoalveolar lavage, phlegm, urine, ejaculate, depending on professional prescription.

General requirements

The quality of taking a sample of biomaterial, its storage, transportation and pre-processing have a great importance for obtaining correct results. PCR research is a direct method, so taking of biological material must be carried out from the location of the infectious process.

Interfering substances

The presence of PCR inhibitors in a sample may cause controversial (uncertain) results. The sign of PCR inhibition is the simultaneous absence of internal control and specific product of amplification.

PCR inhibitors are the presence of hemoglobin in the DNA sample as a result of incomplete removal during the extraction of DNA from a biomaterial sample containing an impurity of blood, as well as the presence of isopropyl alcohol and methyl acetate in the DNA sample as a result of incomplete removal of washing solutions during sample preparation.

The maximum concentration of interfering substances, which do not affect the amplification of the laboratory control sample and internal control: hemoglobin – 0.35 mg/mL DNA sample, isopropyl alcohol – 100 µL/mL DNA sample, methyl acetate – 100 µL/mL DNA sample.

To reduce the count of PCR inhibitors, it is necessary to follow the principles of taking biological material. Suspecting a large count of PCR inhibitors in the sample, it is recommended to choose DNA extraction methods that allow to remove PCR inhibitors from the sample as much as possible.

Sample collection

Cerebrospinal fluid, bronchoalveolar lavage, phlegm, punctate samples:

Cerebrospinal fluid, bronchoalveolar lavage, phlegm, punctate (about 500 µL) is collected in a sterile container, closed tightly and marked.

Biopsy samples:

The biopsy samples (bioptat) are placed into 1.5 mL tube with transport medium intended by the manufacturer for transporting and storing samples of biological material for PCR studies. The tube is closed tightly and marked.

Urine:

The first portion of morning urine in the amount of 10–15 mL is selected for the analysis. It is possible to examine the first portion of urine received 2 or more hours after the previous urination.

The urine is taken into a special dry sterile container with a volume of up to 60 mL, equipped with a hermetically screw-cap.

After the urine collection, container is tightly screwed and marked.

Ejaculate:

Before collecting ejaculate (seminal fluid), sexual abstinence is recommended for 3 days before the examination.

Before collecting the ejaculate, the patient urinates in the toilet, completely emptying the bladder.

After urinating, the patient should wash his hands thoroughly with soap and hold the toilet of the external genitals with soap and water. The penis balanus and the foreskin should be dried with a sterile napkin.

The ejaculate is obtained by masturbation and collected in a sterile container with a volume of up to 60 mL.

The container with ejaculate is hermetically closed and marked.

Transportation and storage of the samples

Samples may be transported and stored at temperatures from 2 °C to 8 °C no more than 24 hours prior to analysis. When it is impossible to deliver the material in the laboratory during the day, a one-time freezing of the material is allowed. The frozen material is allowed to be stored at temperatures from minus 18 °C to minus 22 °C for one month.

In case of usage transport media biological material samples are transported and stored according to the instruction for the transport medium used intended for subsequent sample analysis by PCR.

The detailed description of sampling and sample processing procedures as well as sample storage and transportation requirements cited in **PREP-NA** and **PREP-GS** extraction kits user manuals.

Sample preparation

Cerebrospinal fluid, bronchoalveolar lavage, punctate

It is necessary to perform pretreatment before DNA extraction by the **PREP-GS**, **PREP-NA** kits:

1. Transfer 500 µL of the material into a 1.5 mL tube.
2. Centrifuge the tube at RCF(g) 16000 for 10 minutes at room temperatures (from 18 °C to 25 °C).
3. Remove the supernatant, leaving 50 µL in tube (precipitate + liquid fraction).
4. Add 500 µL of a sterile saline solution to the precipitate.
5. Vortex the tube for 3-5 seconds, then spin for 3-5 seconds.
6. Centrifuge the tube at RCF(g) 16000 for 10 minutes.
7. Remove the supernatant, leaving the volume of precipitate+liquid fraction in the tube that is recommended in the instruction for the DNA extraction kit.

The resulting material is ready for DNA extraction.

Phlegm

It is necessary to perform pretreatment before DNA extraction by the **PREP-GS**, **PREP-NA** kits:

Method 1:

1. Put approximately 500 µL of biological sample into sterile 1.5 mL tube and close it tightly.
2. Add to the sample an equal volume of 10% triple-substituted sodium phosphate x12H_2O, close tightly and mix intensively.
3. Incubate the mixture at 37.0 °C for 18–24 hours, then neutralize with 1.0 M HCl (down to pH 6.8–7.4).
4. Centrifuge1.5 mL tube for 20 minutes at RCF(g) 100.
5. Take out the supernatant into the 5.0 % solution of chloramine for disinfection.
6. Add 500 µL of distilled water to precipitate, mix by pipetting and transfer to the new 1.5 mL tube.
7. Centrifuge the tube at RCF(g) 16000 for 10 minutes.
8. Remove the supernatant, leaving the volume of precipitate+liquid fraction in the tube that is recommended in the instruction for the DNA extraction kit.

The resulting material is ready for DNA extraction.

Method 2:

1. Add mucolysin to the container with sample in the 5:1 ratio (5 parts of mucolysin to 1 part of phlegm), referring to container calibrations.
2. Close the container, mix the container content and incubate it at room temperature for 20–30 minutes, shake the container every 2-3 minutes.

The resulting material is ready for DNA extraction.

It is allowed to store the processed phlegm for one day at temperatures from 2 °C to 8 °C or for a long time at not above minus 16 °C (in case of repeated RNA/DNA extraction necessity).

Biopsy samples

It is necessary to perform pretreatment before DNA extraction by the **PREP-GS**, **PREP-NA** kits:

1. Vortex the tube for 3-5 seconds, then spin down drops for 3-5 seconds.
2. Remove the supernatant.

The resulting material is ready for DNA extraction.

Use only **PREP-NA** and **PREP-GS** extraction kits for DNA extraction from biopsy samples. Using **PREP-NA**, thermostate the tubes at 65 °C for 30 minutes, spin down condensate by centrifuging at RCF(g) 100 for 3–5 seconds and remove supernatant into 1.5 mL new plastic tube. Using **PREP-GS**, add 150 µL of the lysis buffer (without sorbent) into the tubes with biopsy samples, close the tubes tightly, vortex the tubes, then thermostate the tubes for 20 minutes at 50 °C, remove the non-lyzed material, add 20 µL of pre- resuspended sorbent, close the tube and vortex the tubes for 3-5 seconds.

Urine

It is necessary to perform pretreatment before DNA extraction by the **PREP-NA, PREP-GS** kits:

1. Transfer 1.0 mL of the sample to the 1.5 mL tube.
2. Centrifuge the tube at RCF(g) 16000 for 10 minutes.
3. Remove the supernatant completely.
4. Add 1.0 mL of sterile buffered saline to the precipitate.
5. Centrifuge the tube at RCF(g) 16000 for 10 minutes.
6. Remove the supernatant, leaving the volume of precipitate+liquid fraction in the tube that is recommended in the instruction for the DNA extraction kit.

The resulting material is ready for DNA extraction.

Ejaculate

It is necessary to perform pretreatment before DNA extraction by the **PREP-NA, PREP-GS** kits:

1. Transfer 50–100 µL of the liquid sample into the 1.5 mL tube with transport medium (or with 500 µL of sterile buffered saline), vortex the tubes for 5-10 seconds.
2. Centrifuge the tube at RCF(g) 16000 for 10 minutes.
3. Remove the supernatant, leaving the volume of precipitate+liquid fraction in the tube that is recommended in the instruction for the DNA extraction kit.

The resulting material is ready for DNA extraction.

8. PROCEDURE

DNA extraction from biological material

DNA extraction is carried out according to the extraction kit instructions. **PREP-NA** and **PREP-GS** extraction kits are recommended. It is allowed to use any kits of reagents registered as a medical device and recommended by manufacturers for the extraction of DNA from the corresponding types of biomaterial.

Independently of DNA extraction kit used, a negative control sample should go through all stages of DNA extraction. Physiological saline solution can be used as a negative control in volumes as indicated.

Assay procedure for package S

The reagents and tubes should be kept away from direct sun light.

When using package S (R1-P404-S3/4EU), strips, strictly observe the completeness of the strips and caps for them. Do not use the caps for the strips of the other kits!

8.1 Mark tubes with PCR-mix for each test sample, negative control (C-) and positive control (C+).

Example: to test 4 samples, mark 4 tubes for samples, 1 tube for “C-” and 1 tube for “C+”. The resulting number of tubes is 6.

8.2 Vortex the Taq-polymerase solution for 3-5 seconds, then spin for 1-3 seconds.

8.3 Add 10 µL of Taq-polymerase solution into each tube. Avoid paraffin layer break.

8.4 Add one drop (~20 µL) of mineral oil into each tube (not applicable to kits approved for use with Rotor-Gene thermal cycler). Close the tubes.

8.5 Vortex the tubes with samples, "C-" and "C+" for 3-5 seconds and spin down drops for 1-3 seconds.

In case of using **PREP-GS DNA Extraction Kit**. After vortexing centrifuge the tubes with the DNA preparation at RCF(g) 16000 for one minute to precipitate the sorbent. If, after isolation, the supernatant containing the isolated DNA was transferred to new tubes, centrifugation is carried out for 1-3 seconds in a vortex mixer.

Open the tube, add DNA sample (or control sample), then close the tube before proceeding to the next DNA sample to prevent contamination. In case of using tubes in strips, close the strip before proceeding to the next DNA sample to prevent contamination. Close the tube/strips tightly. Use filter tips.

8.6 Add 5.0 µL of DNA sample into corresponding tubes. Do not add DNA into the "C-" and "C+" tubes. Avoid paraffin layer break.

8.7 Add 5.0 µL of negative control (C-) which passed whole DNA extraction procedure into "C-" tube and positive control (C+) into corresponding tube. Avoid paraffin layer break.

8.8 Spin tubes/strips for 3-5 seconds (when using the Rotor-Gene Q thermal cycler, centrifugation is not required).

8.9 Set the tubes/strips into the Real-time Thermal Cycler.

8.10 Launch the operating software for DT instrument[2]. Add corresponding test[3], specify the number and ID's of the samples, positive and negative control samples. Specify the position of the tubes/strips in the thermal unit (see 8.9) and run PCR. See Tables 3, 7.

For use with iQ and Rotor-Gene Q real-time thermal cyclers consult user manual for devices. See Tables 4-7.

Table 3. The PCR program for DTlite and DTprime Thermal Cyclers

Step	Temperature, °C	Min.	Sec.	Number of cycles	Optical measurement	Type of the step
1	80	0	30	1		Cycle
	94	1	30			
2	94	0	30	5		Cycle
	64	0	15		v	
3	94	0	10	45		Cycle
	64	0	15		v	
4	94	0	5	1		Cycle
5	10[1]	…	…	Holding		Holding
[1] – holding at 25°C is allowed						

[2] Please, apply to Operation Manual for DTprime and DTlite Real-Time PCR instruments PART II.
[3] Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website https://www.dna-technology.com/assaylibrary.

Table 4. The PCR program for iCycler iQ thermal cycler (with persistent well factor)

Cycle	Repeats	Step	Dwell time	Setpoint, ºC	PCR/Melt Data Acquisition
1	1				
		1	1 min	80	
		2	1 min 30 sec	94	
2	5				
		1	30 sec	94	
		2	45 sec	64	
3	45				
		1	10 sec	94	
		2	45 sec	64	Real Time
4	…	…	…	10	Storage

Table 5. The PCR program for iCycler iQ thermal cycler (with dynamic well factor)

Cycle	Repeats	Step	Dwell time	Setpoint, ºC	PCR/Melt Data Acquisition
dynamicwf.tmo program					
1	1				
		1	1 min	80	
		2	1 min 30 sec	94	
2	5				
		1	30 sec	94	
		2	45 sec	64	
3	2				
		1	30 sec	80	Real Time
PCR program					
4	45				
		1	10 sec	94	
		2	45 sec	64	Real Time
5	…	…	…	10	Storage

Table 6. The PCR program for Rotor-Gene Q thermal cycler

Cycling	Temperature	Hold time	Cycle repeats
Cycling	80 deg	60 sec	1 time
	94 deg	90 sec	
Cycling 2	94 deg	30 sec	5 times
	57 deg*	15 sec	
Cycling 3	94 deg	10 sec	45 times
	57 deg*	15 sec	
* Take the measurement			

Table 7. Detection channels

Fam (Green)	Hex (Yellow)	Rox (Orange)	Cy5 (Red)	Cy5.5 (Crimson)
Specific product and C+	IC	-	-	-

9. CONTROLS

The **M.tuberculosis - M.bovis REAL-TIME PCR Detection Kit** contains positive control sample. Positive control is a cloned part of the *Mycobacterium* complex *(M.tuberculosis/M.bovis)* genome. It is produced with genetic engineering techniques and characterized by automatic DNA sequencing. The PCR-mix from the kit includes the Internal control (IC). IC is an artificial plasmid intended to assess the quality of PCR performance. To reveal possible contamination a negative control is required.

A negative control sample should go through all stages of DNA extraction. Physiological saline solution can be used as a negative control sample in volumes indicated in supplied instructions.

For **M.tuberculosis - M.bovis REAL-TIME PCR Detection Kit** the test result is considered valid when:

- the exponential growth of the fluorescence level for the specific product is present, in this case the internal control is not taken into account;
- the exponential growth of the fluorescence level for the specific product is absent and for internal control is present.

For **M.tuberculosis - M.bovis REAL-TIME PCR Detection Kit** the test result is considered invalid when the exponential growth of the fluorescence level for the specific product and for internal control is not observed.

If positive control (C+) does **not** express growing fluorescence of the specific product or positive result, it is required to repeat the whole test. It may be caused by inhibitors, operation error or violation of storage and handling.

If negative control (C-) expresses growing fluorescence of the specific product or positive result, all tests of the current batch are considered false. Decontamination is required.

10. DATA ANALYSIS

In case of using DNA-Technology made Real-Time PCR Thermal Cyclers, the analysis is performed automatically. In all other cases, the analysis is based on the presence or absence of specific signal.

In the samples containing *Mycobacterium* complex *(M.tuberculosis/M.bovis)* DNA (specific product), the detecting amplifier registers the expressed growing fluorescence of specific product, the amplification result of the internal control is not taken into account.

In the samples free of *Mycobacterium* complex *(M.tuberculosis/M.bovis)* DNA, the detecting amplifier registers the expressed growing fluorescence of the internal control and its absence for the specific product.

When the unseen expressed growing fluorescence or negative result of both in the specific product and the internal control, the result of amplification is considered as uncertain. It may due to inhibitors, incorrect performance, non-compliance of the amplification temperatures, etc. In this case, amplification, or DNA extraction, or collecting of clinical material are required to be repeated.

In case the result for negative control is defined as positive, the whole experiment should be considered false. The retesting and decontamination are required.

The controls should be also considered to exclude false positive and false negative results (see p. 9 of the current manual). The cutoff Ct values for Rotor-Gene Q thermal cycler are 40 (specific product) and 33 (C+). The result characterized by Ct above this value should be considered doubtful and the whole assay should be repeated.

11. SPECIFICATIONS

a. The analytical specificity of the **M.tuberculosis - M.bovis REAL-TIME PCR Detection Kit** was assessed by bioinformatics analysis using available on-line databases with up-to-date comprehensive genetic information. The specific oligonucleotides used in the test were checked against GenBank database sequences. None of the sequences showed sufficient similarity for unspecific detection.

The samples with *Mycobacterium* complex *(M.tuberculosis/M.bovis)* DNA are to be registered positive for specific product (a fragment of the *Mycobacterium* complex *(M.tuberculosis/M.bovis)* genome). The samples free of *Mycobacterium* complex *(M.tuberculosis/M.bovis)* DNA are to be registered negative for specific product and positive for internal control.

There are not non-specific positive results of amplification of DNA sample in the presence of *Mycobacterium leprae, Streptococcus pneumoniae, Legionella pneumophila, Moraxella catarrhalis*, as well as human DNA in concentrations up to 1.0×10^8 copies / mL of the sample.

b. In a determination of analytical sensitivity, the **M.tuberculosis - M.bovis REAL-TIME PCR Detection Kit** demonstrated the ability to reproducibly detect 1 or more colony forming units (CFU) per PCR reaction.

Sensitivity is 5 copies of *Mycobacterium* complex *(M.tuberculosis/M.bovis)* DNA per amplification tube. Sensitivity is determined by the analysis of serial dilutions of the laboratory control sample (LCS). 94 tests were made for each concentration.

The concentration of LCS, copies per amplification tube	Number of repetitions	Number of positive results	% of positive results
5.0	94	93	98.9
2.0	94	89	94.6
0.5	94	49	52.1
0.0	94	0	0.0

Sensitivity of *Mycobacterium* complex *(M.tuberculosis/M.bovis)* DNA in the sample depends on the sampling and the final volume of the extracted DNA (elution volume).

Sensitivity of 5 copies per amplification tube corresponds to the following values of the DNA concentration of *Mycobacterium* complex *(M.tuberculosis/M.bovis)* in case of using DNA extraction kits produced by DNA Technology:

Sample	DNA extraction kits	
	PREP-NA	**PREP-GS**
-cerebrospinal fluid, bronchoalveolar lavage, phlegm, punctate (extracting from 500 µL of sample); - biopsy material; - ejaculate in 500 µL transport medium; - urine (extracting from 1.0 mL of sample)	50 copies /sample	100 copies /sample

The claimed specifications are guaranteed when DNA extraction is performed with **PREP-NA** REF P-002/1EU and **PREP-GS** REF P-003/1EU extraction kits.

12. TROUBLESHOOTING

Table 8. Troubleshooting

	Result	**Possible cause**	**Solution**
C+	-	Operation error PCR inhibition Violation of storage and handling requirements	Repeat whole test Dispose current batch
C-	+	Contamination	Dispose current batch Perform decontamination procedures
IC	Invalid	PCR inhibition	Repeat whole test Resample

If you face to any undescribed issues contact our customer service department regarding quality issues with the kit:

Phone: +7(495)640.16.93

E-mail: hotline@dna-technology.ru

https://www.dna-technology.com/support

13. QUALITY CONTROL

The quality control procedures performed in accordance with ISO 9001:2015 and ISO 13485:2016:

- observation of quality management in manufacturing of IVDD products;
- creation of values for customers;
- maintenance of the best service quality and customer management.

Contact our customer service by quality issues of the **M.tuberculosis - M.bovis REAL-TIME PCR Detection Kit.**

Technical support:

E-mail: hotline@dna-technology.ru

https://www.dna-technology.com

Manufacturer: "DNA-Technology Research & Production", LLC,

142281, Russia, Moscow Region,

Protvino, Zheleznodorozhnaya Street, 20

Phone/fax: +7(495) 640.17.71

E-mail: info@dna-technology.com

https://www.dna-technology.com

Seller: "DNA-Technology" LLC,

117587, Russia, Moscow,

int. ter. Municipal District Chertanovo Severnoye,

Varshavskoye shosse, 125 Zh, building 5, floor 1, office 12

Phone/fax: +7(495) 640.17.71

E-mail: info@dna-technology.com

https://www.dna-technology.com

14. KEY TO SYMBOLS

IVD	*In vitro* diagnostic medical device		Date of manufacture
	Temperature limit		Consult instructions for use
	Contains sufficient for <n> tests	REF	Catalogue number
	Use-by date		Manufacturer
LOT	Batch code		Keep away from sunlight
	Caution	VER	Version
	Do not reuse	CONTROL +	Positive control
NON STERILE	Non-sterile		

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