ECOLI

eSens

CE

IVD

For Professional Use Only

eSens Mycobacterium tuberculosis/bovis/BCG QL PCR kit

REF ES3309B

Instructions for Use

1 INTENDED USE

eSens Mycobacterium tuberculosis/bovis/BCG QL PCR kit is an *in vitro* nucleic acid amplification test for differentiation of the DNA of *Mycobacterium tuberculosis complex* (*MTC*) including the human (*M.tuberculosis*), the bovine (*M.bovis*) and also the vaccine (*M.bovis* BCG) strains in the clinical material and microorganism cultures using real-time fluorescence hybridization detection of amplified products.

NOTE: The results of PCR analysis are taken into account in complex diagnostics of disease.

NOTE: This analysis requires the use of DNA samples extracted from clinical material or microorganism cultures, in which *MTC* DNA was previously detected or quantitatively identified.

2 PRINCIPLE OF PCR DETECTION

Differentiation between the *Mycobacteria* DNA, extracted from the clinical material or microorganism cultures, uses multiprime PCR with simultaneous amplification of the four DNA fragments, three of them act as specific targets, determining one of the strains from the *Mycobacterium tuberculosis complex*: *M.tuberculosis*, *M.bovis* or *M.bovis* BCG, the fourth DNA fragment is the Internal Control. The reaction mix contains fluorescently labeled oligonucleotide probes, which hybridize to the complementary regions of the DNA targets being amplified, leading to the increase of fluorescent intensity. Fluorescent-hybridization detection of amplification products signals is carried out directly during the PCR analysis with the aid of the thermocycler in the “real-time” mode.

eSens Mycobacterium tuberculosis/bovis/BCG QL PCR kit is a qualitative test that uses the Internal Control STI-87 (IC) from **eSens MTC QL PCR kit**. It must be used in the extraction procedure in order to control the extraction process of each individual sample and to identify possible reaction inhibition.

eSens Mycobacterium tuberculosis/bovis/BCG QL PCR kit uses "hot-start", which greatly reduces the frequency of nonspecifically primed reactions. "Hot-start" is guaranteed by the separation of nucleotides and Taq-polymerase using chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

eSens Mycobacterium tuberculosis/bovis/BCG QL PCR kit contains Enzyme UDG to reduce the risk of contamination by amplification products in PCR laboratory.

The results of amplification are registered in the following fluorescence channels:

Table 1

Channel for fluorophore	**FAM**	**JOE**	**ROX**	**Cy5**
DNA-target	DNA *M.tuberculosis*	DNA *M. bovis, M.bovis BCG*	DNA *M. bovis BCG*	DNA IC
Target gene	RD 9	RD 9	RD 1	Artificially synthesized sequence

3 CONTENT

eSens Mycobacterium tuberculosis/bovis/BCG QL PCR kit (ES3309B) includes:

Reagent	**Description**	**Volume, ml**	**Quantity**
PCR-mix-1-FL *MTC*-diff	clear liquid from colorless to light lilac colour	0.28	2 tubes
PCR-mix-2-FRT	colorless clear liquid	0.3	1 tube
Polymerase (TaqF)	colorless clear liquid	0.03	1 tube
Enzyme UDG	colorless clear liquid	0.03	1 tube
Positive Control DNA *MTC*-diff / STI ($C+_{MTC\text{-}diff\ /\ STI}$)	colorless clear liquid	0.1	1 tube
TE-buffer	colorless clear liquid	0.5	1 tube

eSens Mycobacterium tuberculosis/bovis/BCG QL PCR kit is intended for 55 reactions (including controls).

4 ADDITIONAL REQUIREMENTS

- Disposable powder-free gloves and a laboratory coat.
- Pipettes (adjustable).
- Sterile RNase-free pipette tips with aerosol filters (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with a rotor for 2-ml reaction tubes.
- PCR box.

- Real-time instruments (for example, Rotor-Gene Q (QIAGEN, Germany), CFX 96 Touch, CFX 96 Opus (Bio-Rad, USA), QuantStudio 5 (Thermo Fisher Scientific), or equivalent).
- Disposable polypropylene PCR tubes:
  a) 0.2-ml PCR tubes with optical transparent domed or flat caps if a plate-type instrument is used;
  b) 0.2-ml PCR tubes with flat caps or strips of four 0.1-ml Rotor-Gene PCR tubes if a rotor-type instrument is used.
- Refrigerator with temperature range from 2 to 8 °°C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir for used tips.

5 GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol filters and use a new tip for every procedure.
- Store all extracted positive material (specimens, controls and amplicons) away from all other reagents and add it to the reaction mix in a distantly separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable protective gloves and laboratory cloths, and protect eyes while samples and reagents handling. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use a kit after its expiration date.
- Dispose of all specimens and unused reagents in accordance with local regulations.
- Samples should be considered potentially infectious and handled in biological cabinet in compliance with appropriate biosafety practices.
- Clean and disinfect all samples or reagents spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.
- Avoid inhalation of vapors, samples and reagents contact with the skin, eyes, and mucous membranes. Harmful if swallowed. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice if necessary.
- Safety Data Sheets (SDS) are available on request.
- Use of this product should be limited to personnel trained in DNA amplification techniques.
- Workflow in the laboratory must be one-directional, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where the previous step was performed.

⚠ Some components of this kit contain sodium azide as a preservative. Do not use metal tubing for reagent transfer.

6 SAMPLING AND HANDLING

eSens Mycobacterium tuberculosis/bovis/BCG QL PCR kit is intended as a supplement test for **eSens MTC QL PCR kit** and is used for analysis of the DNA previously extracted from the clinical material. Please, consult the **eSens MTC QL PCR kit** *Instructions Manual* for further information on sampling and pre-treatment procedures.

7 WORKING CONDITIONS

eSens Mycobacterium tuberculosis/bovis/BCG QL PCR kit should be used at 18–25 °C.

8 PROTOCOL

8.1 DNA extraction

Any commercial nucleic acid extraction kit, if IVD-CE validated for the indicated specimen types, could be used.

eSens Mycobacterium tuberculosis/bovis/BCG QL PCR kit is intended as a supplement test for **eSens MTC QL PCR kit** and is used for analysis of the DNA previously extracted from the clinical material. The **Internal Control STI-87 (IC)** used for this experiment is also taken from **eSens MTC QL PCR kit**, thus consult the **eSens MTC QL PCR kit** *Instructions Manual* for further information.

NOTE: Extract the DNA according to the manufacturer's protocol.

8.2 Preparing PCR

8.2.1 Preparing tubes for PCR

The type of tubes depends on the PCR instrument used for analysis. Use disposable filter tips for adding reagents, DNA and control samples into tubes.

NOTE: Components of the reaction mix should be mixed directly before use.

1. Prepare the required number of tubes for amplification including the number of test samples and control samples (2 amplification controls).
2. Prepare the reaction mix in 1.5 ml tube for each reaction:

   10 µl of **PCR-mix-1-FL *MTC*-diff**
   5 µl of **PCR-mix-2-FRT**
   0.5 µl of **Polymerase (TaqF)**
   0.5 µl of **Enzyme UDG**

   Vortex the tubes until the drops are settled from the lids.

3. Add **15 µl** of the prepared reaction mix to each tube.
4. Prepare the tubes with extracted DNA, in which *Mycobacterium tuberculosis complex* (*MTC*) DNA was previously found with the aid of **eSens MTC QL PCR kit**. In case of the need to store the tubes, vortex the tubes and then centrifuge at 10,000 rpm for 2 min.
5. To the prepared tubes add **10 µl** of **DNA samples**.

NOTE: DNA samples obtained previously can be used for analysis, given that it was stored during 1 week at a temperature from 2 to 8°C, during 1 year at a temperature not more than minus 16°C, any period of storage at a temperature not more than minus 68°C.

6. Carry out the control amplification reactions:

NCA	–	Add **10 µl** of **TE-buffer** to the tube labeled NCA (Negative Control of Amplification)
C+	–	Add **10 µl** of **Positive Control DNA *MTC*-diff / STI (C+$_{MTC\text{-}diff\,/\,STI}$)** to the tube labeled C+

NOTE: It is recommended to sediment drops from walls of tubes by short vortexing (1–3 s) before placing them in the thermocycler.
For carrying out decontamination of reaction mix incubate prepared tubes at room temperature for 10–30 min.

8.2.2 Amplification

1. Create a temperature profile on your instrument as follows:

Table 2

"95-65-72 MTC" amplification program

Rotor-type Instruments (e.g Rotor-Gene Q or equivalent)		**Plate-type Instruments** (e.g CFX 96 Touch, CFX 96 Opus, QuantStudio 5 or equivalent.)	
Step	**Temperature, °C**	**Time**	**Cycles**
1	**95**	**15 min**	**1**
2	**95**	**15 s**	**5**
	65	**30 s**	
	72	**15 s**	
3	**95**	**15 s**	**40**
	65	**30 s**	
	72	**15 s**	

NOTE: If Rotor-Gene Q (QIAGEN, Germany) instrument is being used, then rotor's well №1 must be filled with any sample tube from this experiment, except for an empty one. If one rotor is loaded with the sample tubes that are being analyzed by different reagents kits, then the loading order of the sample tubes will be the following: first load the tubes with reagents for differentiation, then load the tubes for quantitative detection, lastly load tubes for detection of *Mycobacterium tuberculosis complex*.

Fluorescent signal is detected in the channels for the **FAM, JOE/HEX, ROX** and **Cy5** fluorophores.

2. Adjust the fluorescence channel sensitivity.
3. Insert tubes into the reaction module of the device.
4. Run the amplification program with fluorescence detection.
5. Analyze results after the amplification program is completed.

8.3 Instrument Settings

Test settings for rotor-type instruments

Channel	**Calibrate/Gain Optimisation**	**Threshold**	**Dynamic tube**	**Slope Correct**	**More Settings/ Outlier Removal**
FAM/Green	from 5 Fl to 10 Fl	0.03	On	On	10%
JOE/Yellow	from 5 Fl to 10 Fl	0.05	On	On	10%
ROX/Orange	from 5 Fl to 10 Fl	0.05	On	On	10%
Cy5/Red	from 5 Fl to 10 Fl	0.05	On	On	30%

Test settings for plate-type instruments

Note: Set ***Ramp Rate* 2,5 °C/s** by clicking the ***Step Options*** button for each step of cycling.

Channel	**Threshold**
FAM, HEX/JOE, ROX, Cy5	The threshold line for each channel is set at the level of 10-20% of maximum, fluorescence obtained for the C+ in the last amplification cycle.

9 DATA ANALYSIS

Analysis of results is performed by the software of the real-time PCR instrument used by measuring fluorescence signal accumulation in four channels.

- The signal of the Internal Control DNA amplification product is detected in the channel for the **Cy5** fluorophore.
- The signal of human mycobacteria DNA (*M.tuberculosis*) is detected in the channel for the **FAM** fluorophore.
- The signal of bovine mycobacteria DNA (*M.bovis* or *M.bovis* BCG) is detected in the channel for the **JOE** fluorophore.
- The signal of only the vaccine strain (*M.bovis* BCG) is detected in the channel for the **ROX** fluorophore.

Results are interpreted by the interception (or absence of interception) of the fluorescence curve with the threshold line set at the specific level that corresponds to the presence (or absence) of a *Ct (Cp)* value of the DNA sample in the corresponding column of the results grid.
Principle of interpretation is the following:

- The result is considered to be **positive (+)** if the fluorescence curve has a typical sigmoid form and intercepts the threshold line in the area of exponential fluorescence growth.
- The result is considered to be **negative (–)** if the fluorescence curve does not have a typical sigmoid form and it does not intercept the threshold line, therefore *Ct* (*Cp*) value is absent.
- The result is considered to be **equivocal (>*Ct*)** if the value of the threshold is higher than presented.
- The **invalid** result in a channel is formed of the plurality of the negative values of that channel and negative or equivocal result in **Cy5** channel, which was reproduced after secondary sample analysis.

Positive result in the **FAM** channel implies that human strain (*M.tuberculosis*) is detected.

Positive result in the **JOE** channel implies that bovine strains (*M.bovis* and *M.bovis* BCG) are detected.

Positive result in the **ROX** channel implies that bovine vaccine strain (*M.bovis* BCG) is detected.

Positive result in **Cy5** channel confirms adequate procession of extraction and amplification steps.

NOTE: Results interpretation from *Mycobacterium tuberculosis complex* (MTC) strain differentiation is conducted independently for each channel.
The reagents kit allows detection of specified strains from *M.tuberculosis complex* in mixes (for example, *M.tuberculosis* and *M.bovis* mix or *M.tuberculosis* and *M.bovis* BCG mix). An exception to that is *M.bovis* and *M.bovis* BCG mix, due to the fact that *M.bovis* BCG belongs to the bovine *MTC* strain (*M.bovis*).

The result of the analysis is considered reliable only if the results obtained for Positive and Negative Controls of amplification are correct (see Table 3).

Table 3

Results for controls

Control	Stage for control	*Ct* value in the channel for fluorophore			
		FAM	JOE	ROX	Cy5
NCA	PCR	–	–	–	–
C+	PCR	+	+	+	+

Table 4

Results interpretation for analyzed clinical samples or microorganism cultures

DNA	FAM	JOE	ROX	Cy5	Result	Result interpretation
M.tuberculosis	**+**	**Any result possible***	**Any result possible**	**+/–**	**Detected**	*M.tuberculosis DNA* (human species) is detected
	–	**Any result possible**	**Any result possible**	**+**	**Not detected**	*M.tuberculosis* DNA (human species) is not detected
	>*Ct*	**Any result possible**	**Any result possible**	**+**	**Equivocal**	*M.tuberculosis* DNA (human species) detection is equivocal. It is recommended to repeat sampling and analysis of material
	–/>*Ct*	**Any result possible**	**Any result possible**	**– />*Ct***	**Invalid**	*M.tuberculosis* DNA (human species) – invalid result. It is recommended to repeat sampling and analysis of material
M.bovis	**Any result possible**	**+**	**–**	**+/–**	**Detected**	*M.bovis* DNA (bovine species) is detected
	Any result possible	**–**	**Any result possible**	**+**	**Not detected**	*M.bovis* DNA (bovine species) is not detected
	Any result possible	**>*Ct***	**–**	**+**	**Equivocal**	*M.bovis* DNA (bovine species) detection is equivocal. It is recommended to repeat sampling and analysis of material
	Any result possible	**–/>*Ct***	**Any result possible**	**– />*Ct***	**Invalid**	*M.bovis* DNA (bovine species) – invalid result. It is recommended to repeat sampling and analysis of material

***M.bovis* BCG**	**Any result possible**	**Any result possible**	**+**	**+/–**	**Detected**	*M.bovis* BCG DNA (vaccine strain) is detected
	Any result possible	**+/–**	**–**	**+**	**Not detected**	*M.bovis* BCG DNA (vaccine strain) is not detected
	Any result possible	**Any result possible**	**>*Ct***	**+**	**Equivocal**	*M.bovis* BCG DNA (vaccine strain) – detection is equivocal. It is recommended to repeat sampling and analysis of material
	Any result possible	**Any result possible**	**–/>*Ct***	**– />*Ct***	**Invalid**	*M.bovis* BCG DNA (vaccine strain) – invalid result. It is recommended to repeat sampling and analysis of material

***** Any result possible– the analysis result can be either: positive (+), negative (–) or equivocal (>*Ct*).

Results interpretation principle:

- If a positive result **(+)** is obtained in one of the channels **FAM**, **JOE** or **ROX**, along with a positive or negative result in channel **Cy5**, then either ***M.tuberculosis, M.bovis*** or ***M.bovis* BCG** is **detected** in the tested sample, respectively. In case of ***M.bovis* BCG** detection, a positive signal may also be registered in the **JOE** channel.
- If a negative result **(–)** is obtained in one of the channels **FAM**, **JOE** or **ROX**, along with a positive result in channel **Cy5**, then either ***M.tuberculosis, M.bovis*** or ***M.bovis* BCG** is **not detected** in the tested sample, respectively.
- If a positive result **(+)** is obtained in several of the channels **FAM**, **JOE** or **ROX**, then a mix of different strains from ***M.tuberculosis complex*** is **detected**.

NOTE: An exception to this is a combination of positive signals in JOE and ROX channels, indicating the detection of ***M.bovis* BCG** in the tested sample. Therefore, if the tested sample contains a mix of ***M.bovis* BCG** and ***M.bovis***, then the sample is identified as ***M.bovis* BCG**.

- If an equivocal result **(>*Ct*)** is obtained in one of the channels **FAM**, **JOE** or **ROX**, along with a positive result in channel **Cy5**, then the DNA sample amplification needs to be repeated. If a significant result is not obtained, repeat extraction and DNA amplification steps using the original sample. Obtaining an analogous result (>*Ct*) is interpreted as an equivocal result**.**
- If a negative or equivocal result is obtained in channels **FAM**, **JOE** or **ROX**, along with a negative or equivocal result in channel **Cy5**, the result is suggested to be invalid. In this case, repeat the amplification of the sample. If a valid result is not obtained, repeat **extraction and DNA amplification steps using the original sample. Obtaining an analogous result (–/>*Ct*) is interpreted as invalid.**

NOTE: The validity of results is assessed independently for each channel.

10 TROUBLESHOOTING

The results of analysis are not taken into account in the following cases:

- If the positive signal is not detected in one or more detection channels for positive control of PCR (C+). In this case, repeat the C+ amplification. If the repeated procedure gives no positive signal, then the reagents kit is not suitable for further use.
- If the positive signal is detected in one or more detection channels for negative control of PCR (NCA). In this case, repeat the amplification no less than for 5 samples of negative control of PCR (NCA). If the repeated procedure still gives a positive single in at least one sample, the reagents kit is not suitable for further use.

11 TRANSPORTATION

eSens Mycobacterium tuberculosis/bovis/BCG QL PCR kit should be transported at 2–8 °C for no longer than 5 days.

12 STABILITY AND STORAGE

All components of the **eSens Mycobacterium tuberculosis/bovis/BCG QL PCR kit** are to be stored at 2–8 °C when not in use. All components of the **eSens Mycobacterium tuberculosis/bovis/BCG QL PCR kit** are stable until the expiry date stated on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

NOTE: PCR-mix-1-FL *MTC*-diff, PCR-mix-2-FRT, polymerase (TaqF) and Enzyme UDG are to be stored at the temperature from minus 24 to minus 16 °C

NOTE: PCR-mix-1-FL *MTC*-diff is to be kept away from light

13 SPECIFICATIONS

13.1 Sensitivity

Table 5

Clinical material	DNA extraction kit	Sensitivity, mb/ml			
		eSens MTC QL PCR kit	eSens Mycobacterium tuberculosis /bovis/BCG QL PCR kit	eSens MTC QL PCR kit	eSens Mycobacterium tuberculosis/ bovis/BCG QL PCR kit
		***M.tuberculosis* (H37 Ra strain)**		***M.bovis* BCG (*M.bovis* BCG strain)**	
PBS-buffer	RIBO-prep ePure TB DNA extraction kit	$5x10^2$	$1x10^3$	$1x10^3$	$1x10^3$
Urine		$1x10^3$	$5x10^3$	$1x10^3$	$1x10^3$
Sputum*, BAL/BWF		$5x10^2$	$1x10^3$	$1x10^3$	$1x10^3$
PBS-buffer	DNA-sorb-B ePure TB DNA extraction kit	$5x10^2$	$1x10^3$	$1x10^3$	$1x10^3$
Sputum*, BAL/BWF		$5x10^2$	$5x10^3$	$1x10^3$	$5x10^3$
Urine		$1x10^3$	$5x10^3$	$1x10^3$	$5x10^3$

***** Concentration in 1 ml of sputum, treated with **Mucolysin** (180-CE) reagent for pre-treatment of mucous material.

13.2 Specificity

The analytical specificity of **eSens Mycobacterium tuberculosis/bovis/BCG QL PCR kit** is ensured by the selection of specific primers and probes as well as stringent reaction conditions. The primers and probes have been checked for possible homologies to all sequences published in gene banks by sequence comparison analysis.

The analytical specificity of **eSens Mycobacterium tuberculosis/bovis/BCG QL PCR kit** was assessed during strain analysis of mycobacteria from *M.tuberculosis complex*, nontuberculous mycobacteria, and also other microorganism strains that induce illnesses in similar locations.

In order to define the analytical specificity on different microorganism strains (with concentration no less than $5x10^8$ mb/ml), 116 control strains or clinical isolators were tested. Five of them were included into *M.tuberculosis complex*, 31 strains were nontuberculous mycobacteria (NTM), and the remaining 80 strains belong to other genii and families. The analytical specificity was assessed based on the absence of the positive DNA amplification result for samples not included in *M.tuberculosis complex*, and also based on the presence of the positive DNA amplification result in complementary detection channel for differentiation of mycobacterial strains of the *M.tuberculosis complex*.
List of reference strains:

- Mycobacteria, that are included in *Mycobacterium tuberculosis complex*: *M.tuberculosis, M.bovis, M.bovis* BCG-*1,* etc.

- Nontuberculous mycobacteria: *M.avium, M.paratuberculosis, M.xenopi, M.gordonae, M.ulcerans, M.phlei, M.intracellulare, M.kansasii, M.fortuitum.*
- Bacteria belonging to other genii and families: *Brucella, Campylobacter, Chlamydophila, Cryptococuss, Enterobacter, Enterococcus, E.coli, Klebsiella, Listeria, Moraxella, Neisseria, Pantoea, Pasteurella, Proteus, Pseudomonas, Salmonella, Shigella, Staphylococcus, Streptococcus.*

The clinical specificity of **eSens Mycobacterium tuberculosis/bovis/BCG QL PCR kit** was confirmed in laboratory clinical trials.

14 QUALITY CONTROL

The production process, including batch release, is carried out in accordance with an established quality management system certified according to ISO 13485.

15 KEY TO SYMBOLS USED

REF	Catalogue number	⚠	Caution
LOT	Batch code	Σ	Contains sufficient for <n> tests
IVD	*In vitro* diagnostic medical device		Use-by Date
VER	Version		Consult instructions for use
	Temperature limit		Keep away from sunlight
	Manufacturer	**NCA**	Negative control of amplification
	Date of manufacture	**C-**	Negative control of extraction
EC REP	Authorized representative in the European Community	**C+**	Positive control of amplification
		IC	Internal control

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
01_04/2022		

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