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For Professional Use Only

eSens MTC QL PCR kit

REF ES3305B

Instructions for Use

1 INTENDED USE

eSens MTC QL PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Mycobacterium tuberculosis (MBT)* DNA – *Mycobacterium tuberculosis complex (MTC),* including *M.tuberculosis, M.bovis, M.africanum, M.microti, M.canetti, M.pinipedii* – in clinical material, cultures of microorganisms and environmental objects by using end-point hybridization-fluorescence detection of amplified products.

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

2 PRINCIPLE OF PCR DETECTION

Mycobacteria tuberculosis detection by the polymerase chain reaction (PCR) is based on the amplification of a pathogen genome specific region using specific *Mycobacteria tuberculosis* primers. In the real-time PCR, the amplified product is detected with the use of fluorescent dyes. These dyes are linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. The real-time PCR monitoring of fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run.

eSens MTC QL PCR kit is a qualitative test that contains the Internal Control (Internal Control STI-87). 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 MTC QL PCR kit uses “hot-start”, which greatly reduces the frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by separation of nucleotides and Taq-polymerase by using a wax layer or a chemically modified polymerase (TaqF). Wax melts and reaction components mix only at 95 °C. Chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

eSens MTC QL PCR kit includes enzyme uracil-DNA glycosylase (UDG) to reduce the risk of contamination.

For optimization of *Mycobacteria tuberculosis* research report, an integrated procedure of DNA extraction for quantitative detection, identification to species, and determination of resistance to antitubercular therapy can be carried out.

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

Table 1

Channel for fluorophore	FAM	JOE
DNA-target	DNA *M. tuberculosis* complex	DNA IC
Target gene	IS 6110	Artificially synthesized sequence

3 CONTENT

eSens MTC QL PCR kit (ES3305B) includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-1-FRT *MTC*	clear liquid from colorless to light lilac colour	0.28	2 tubes
PCR-buffer-Flu	colorless clear liquid	0.28	1 tube
Polymerase (TaqF)	colorless clear liquid	0.03	1 tube
Enzyme UDG	colorless clear liquid	0.03	1 tube
Positive Control DNA *MTC* / STI ($C+_{MTC/STI}$)	colorless clear liquid	0.1	1 tube
TE-buffer	colorless clear liquid	0.5	1 tube
Negative Control (C-)*	colorless clear liquid	1.6	1 tube
Internal Control STI-87 (IC)**	colorless clear liquid	1.0	1 tube
RNA-buffer***	colorless clear liquid	1.2	1 tube

***** must be used in the extraction procedure as Negative Control of Extraction.

****** add **10 µl** of **Internal Control STI-87 (IC)** during the DNA extraction procedure directly to the sample/lysis mixture.

******* used for elution during DNA extraction.

eSens MTC QL PCR kit is intended for 55 reactions (including controls).

4 ADDITIONAL REQUIREMENTS

- Reagent for pretreatment of viscous fluids (sputum, aspirates).
- Homogenizer is recommended to use for tissue material homogenization.
- Sterile stainless steel balls with 5 mm and 7 mm diameter for tissue material homogenization.
- Sterile porcelain or glass beads with 3-5 mm diameter for *sputum* homogenization and 3 mm diameter for tissue material homogenization.
- DNA extraction kit.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).

- Sterile pipette tips with aerosol barriers (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with 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 (flat caps, nonstriped) for 36-well rotor if a rotor-type instrument is used;
  b) 0.2-ml PCR tubes (domed caps) if a plate-type instrument is used.
- Refrigerator for 2–8 °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

Sampling

6.1 Bronchoalveolar lavage (BAL) and *bronchoalveolar lavage fluid* (*BALF*) and *liquor*.

Bronchoalveolar lavage (BAL) and bronchoalveolar lavage fluid (BALF) and liquor., are collected to disposable hermetically screwed polypropylene vessels (for preventing adhesion of the cells on their internal surface) with a volume no less than 5 ml.

6.2 Sputum and urine.

Sputum and urine (medium portion) is collected to disposable graduated screwed vessels with a wide neck with a volume no less than 50 ml.

6.3 Fasting morning whole blood and *pleural fluid*.

Fasting morning whole blood and pleural fluid is collected to tubes (for example, Vacuette®) with EDTA spraying or its solute. Close the tube and turn it upside down and back several times.

6.4 Menstrual blood.

Menstrual blood is collected to dry disposable test tubes using a Kafka cap.

6.5 Synovial fluid.

Synovial fluid is collected to dry disposable test tubes.

6.6 Prostate gland secretion.

Prostate gland secretion is collected to sterile disposable 1.5-ml tubes after massage of the prostate gland. If, after massage of prostate gland, it is impossible to get the secretion, use the first portion of urine, which contains the prostate gland secretion.

6.7 Tissue (biopsy, surgical).

Tissue (biopsy, surgical) material is collected to tubes (for example, Vacuette®) with EDTA spraying or to disposable tubes with 0.2 ml of sterile saline or PBS.

6.8 Paraffin units.

Paraffin units are cut by using microtome or cut out a fragment of tissue by disposable scalpel. Then remove paraffin by using xylene, remove xylene by series of ablution with decrease of ethanol concentration (similarly to standard histolytic conducting).

6.9 Cultures of microorganisms.

Cultures of microorganisms grown on selective solid nutrient media for *Mycobacteria tuberculosis* are collected to glass tubes as working with turbidity standard by resuspending in saline. Cultures of microorganisms grown on selective liquid nutrition media are used in original vial.

6.10 Washing fluids from environmental objects.

Washing fluids from environmental objects are collected with a tent with a wad wetted in saline. The square of washing from flat surface is 5-10 cm^2. The working part of the tent is to be transferred to the 1.5-ml tube with 0.5 ml of sterile saline. The top of the tent is to be broken and removed.

The samples (except for urine) are to be stored at 2–8 °C for 3 days, at ≤ –16 °C for 1 year. For archiving (more than 1 year), store the samples at ≤ –68 °C.

Urine can be stored at 2–8 °C for no longer than 6 hours. Freeze urine for a long storage. Double freezing-thawing of the clinical material is allowed.

NOTE: Do not freeze blood.

Transport the samples in thermocontainer for no more than 3 days.

Pretreatment

6.11 BAL or BALF.

Mix BAL or BALF by turning upside down and back. Transfer 1 ml of the sample to a 1.5-ml Eppendorf tube using a pipette with a tip with aerosol barrier, mark it, and centrifuge at 10000 g for 10 min. Carefully remove the supernatant using a tip with aerosol barrier and leaving about 100 µl of the sample.

6.12 Sputum.

Add **Mucolysin** (180-CE) to the vessel with *sputum* (5 : 1, v/v) and then add and 3-5 sterile porcelain or glass beads to this mixture. Stir the vessel periodically for 20–30 min. Transfer 100 µl of the sample to 1.5-ml tube Eppendorf using a pipette with a tip with aerosol barrier and mark it.

6.13 Urine.

Mix urine by turning the vessel upside down and back. Using a pipette with a tip with aerosol barrier, transfer 5–10 ml of the sample to a screwed tube, mark it, and centrifuge at 10000 g for 10 min (or at 3000 g for 20 min). Carefully remove the supernatant using a tip with aerosol barrier and leaving about 100 µl of the sample (if the pellet is visible, remove the supernatant leaving just a pellet).

6.14 Synovial fluid.

Add **Mucolysin** (180-CE) to the vessel with synovial fluid (1:1, v/v). Stir the vessel periodically for 20-30 min.

6.15 Cultures of microorganisms.

Resuspend cultures of microorganisms grown on solid nutrient medium (SNM) in a sterile saline or PBS using turbidity standard No. 5 ($5x10^8$ microbial bodies per 1 ml (m.b./ml)) or McFarland No. 0.5, 1 or 2. Use 5 µl of this suspension. Take a 1-ml aliquot of cultures of microorganisms grown on liquid nutrient medium (LNM) and centrifuge it at 1000 g for 10 min. Discard the supernatant.

6.16 Washing fluids from environmental objects.

Use 100-µl aliquots of washing fluids from environmental objects.

Table 2

The samples volume for treatment and DNA extraction

Material	Aliquot volume for treatment	Aliquot volume for DNA extraction
Sputum	All sample	0.1 ml
BAL or BALF	1 ml	0.1 ml
Urine	5–10 ml	0.1 ml
Liquor	1 ml	0.1 ml
Synovial fluid	1 ml	0.1 ml
Prostate gland secretion	1 ml	0.1 ml
SNM	1.5-6 x 10^8 m.b./ml	0.05 ml
LNM	1 ml	0.1 ml
Blood	0.1 ml	
Menstrual blood	0.1 ml	
Tissue	10–25 µl	
Washing fluids from environmental objects	0.1 ml	

NOTE: It is necessary to prevent the repeated sample extraction and reserve the sample aliquot in accordance with storage regulations.

7 WORKING CONDITIONS

eSens MTC 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.

Ecoli Dx, s.r.o. recommends:

- For the manual extraction
  - **DNA-sorb-B** (K1-2-100-CE) - for clinical material, cultures of microorganisms, and environmental objects.
  - **RIBO-prep** (K2-9-Et-100-CE) - for clinical material, cultures of microorganisms, and environmental objects).

- For the automatic extraction
  - **ePure TB DNA Extraction Kit** (E2008)
  - **ePure Bacterial DNA Extraction Kit** (E2006)

NOTE: Extract the DNA according to the manufacturer's protocol.
The DNA extraction for each sample is carried out in the presence of **Internal Control STI-87 (IC).**

NOTE: In case of DNA extraction from the urine add the **Lysis Solution** into the tubes with material pellet, resuspend it using individual tip for each sample and transfer into the 1.5-ml Eppendorf tubes.

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.

The total reaction volume is **25 µl**, the volume of DNA sample is **10 µl**.

Use disposable filter tips for adding reagents, DNA and control samples into tubes.

NOTE: Before starting work, thaw and thoroughly vortex all reagents of the kit. Make sure that there are no drops on the caps of the tubes. All components of the reaction mixture should be mixed immediately before use.

1. Take the required number of tubes for amplification of the DNA obtained from clinical and control samples.
2. For N reactions, add to a new tube:

   10*(N+1) µl of **PCR-mix-1-FRT *MTC*,**
   5*(N+1) µl of **PCR-buffer-Flu,**
   0.5*(N+1) µl of **polymerase (TaqF),**
   0.5*(N+1) µl of **enzyme UDG.**

   Vortex the tube, then centrifuge it briefly

3. Transfer **15 µl** of the reaction mixture to each tube.
4. Add **10 µl** of **DNA samples** obtained at the DNA extraction stage from clinical or control samples.
5. 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* / STI** to the tube labeled C+ (Positive Control of Amplification).
C–	– Add **10 µl** of **the sample extracted from the Negative Control (C–) reagent** to the tube labeled C– (Negative control of Extraction).

NOTE: For carrying out decontamination of the reaction mixture 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 3

«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**	**Temperature, °C**	**Time**	**Cycles**
1	**95**	**15 min**	**1**	**95**	**15 min**	**1**
2	**95**	**15 s**	**5**	**95**	**15 s**	**5**
	65	**30 s**		**65**	**30 s**	
	72	**15 s**		**72**	**15 s**	
3	**95**	**15 s**	**40**	**95**	**15 s**	**40**
	65	**30 s**		**65**	**30 s**	
	72	**15 s**		**72**	**15 s**	

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

2. Adjust the fluorescence channel sensitivity.
3. Insert tubes into the reaction module of the device.

NOTE: It is recommended to sediment drops from walls of tubes by short centrifugation (1–3 s) before placing them into the instrument.

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	Threshold	Slope Correct	More Settings/ Outlier Removal
FAM/Green	0.03	on	10 %
JOE/Yellow	0.05	off	15 %

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	30
JOE/HEX	45

9 DATA ANALYSIS

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

- The signal of the IC DNA amplification product is detected in the channel for the JOE fluorophore,
- The signal of the *Mycobacterium tuberculosis complex* DNA amplification product is detected in the channel for the FAM fluorophore.

The results are interpreted by the crossing (or not-crossing) of the fluorescence curve with the threshold line set at the specific level that corresponds to the presence (or absence) of a *Ct* value of the DNA sample in the corresponding column of the results grid.

Principle of interpretation is specified in the Table 4.

Table 4

Interpretation of amplification results

***Ct* value in the channel for fluorophore**		**Result validity**	**Interpretation**
FAM	**JOE**		
< boundary value	< boundary value or Absent	Valid	*M.tuberculosis complex* is **detected**
Absent	< boundary value	Valid	*M.tuberculosis complex* is not **detected**
Absent or > boundary value	Absent or > boundary value	Invalid	**Invalid** result (repeat material sampling and assay)
> boundary value	< boundary value	Invalid	**Equivocal** result (repeat material sampling and assay)

- If the result is positive in the channel for FAM fluorophore (the *Ct* value does not exceed the specified boundary *Ct* value) and the result is positive (the *Ct* value does not exceed the specified boundary *Ct* value) or negative (*Ct* value is absent) in the channel for JOE fluorophore - the result is **valid**, *Mycobacterium tuberculosis* DNA **is detected**.
- If the result is negative (*Ct* value is absent) in the channel for FAM fluorophore and the result is positive (the *Ct* value does not exceed the specified boundary *Ct* value) in the channel for JOE fluorophore - the result is **valid**, *Mycobacterium tuberculosis* DNA **is not detected**.
- If the result is negative (*Ct* value is absent) or the *Ct* value exceeds the specified boundary *Ct* value in the channels for JOE and FAM fluorophores, the result is **invalid**. It is necessary to repeat amplification. If the result is the same, repeat the assay beginning from the DNA extraction. If the result is the same again, it is considered to be **invalid**. In this case, it is recommended to repeat material sampling and assay.
- If the *Ct* value exceeds the specified boundary *Ct* value in the channel for FAM fluorophore and the result is positive (the *Ct* value does not exceed the specified boundary *Ct* value in the channel for JOE fluorophore, the result is **invalid**. It is necessary to repeat amplification. If the result is the same, repeat the assay beginning from the DNA extraction. If the result is the same again, it is considered to be **equivocal**. In this case, it is recommended to repeat material sampling and assay

The result of the analysis is considered reliable only if the results obtained for the Positive and Negative Controls of amplification as well as for the Negative Control of extraction are correct (see Table 5 and Table 6).

Table 5

Results for controls

Control	Stage for control	*Ct* value in the channel for fluorophore	
		FAM	JOE
C–	DNA extraction	Absent	< boundary value
NCA	PCR	< boundary value	< boundary value
C+	PCR	Absent	Absent

Table 6

Boundary *Ct* values

Sample	Rotor-type instrument		Plate-type instrument	
	Channel for fluorophore			
	FAM	JOE	FAM	JOE
C+	<35	< 33	< 36	< 34
C-	Ct is absent	< 35	Ct is absent	< 36
Test samples	≤ 38	≤ 38	≤ 38	≤ 38

10 TROUBLESHOOTING

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

1. If the positive signal is absent for the Positive Controls of amplification in the channels for FAM or JOE fluorophores, it may suggest that the wrong amplification program was chosen, or other mistakes were made in the amplification stage. The PCR amplification should be repeated for all negative samples. If the same result is obtained again, the PCR analysis (beginning with the DNA extraction stage) should be repeated for such samples.
2. If the positive signal is detected for the Negative Control of Amplification (NCA) and/or Negative Control of Extraction (C–) in the channel for FAM fluorophore, it indicates contamination of reagents or test samples. The PCR analysis (beginning with the DNA extraction stage) should be repeated for all positive samples.
3. If the positive signal is absent for the Negative Control of Extraction (C–) in the channel for FAM fluorophore, it indicates the mistakes made in the DNA extraction stage. In this case the PCR analysis (beginning with the DNA extraction stage) should be repeated for the samples.

11 TRANSPORTATION

eSens MTC 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 MTC QL PCR kit** are to be stored at 2–8 °C when not in use (except for polymerase (TaqF), enzyme UDG, and PCR-mix-1-FRT *MTC*). All components of the **eSens MTC QL PCR kit** are stable until the expiration date on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

NOTE: Polymerase (TaqF), enzyme UDG, and PCR-mix-1-FRT MTC are to be stored at the temperature from minus 24 to minus 16 °C.

NOTE: PCR-mix-1-FRT *MTC* is to be kept away from light.

13 SPECIFICATIONS

13.1 Sensitivity

DNA extraction kit	**Material**	**Sensitivity, mb/ml**
		***M.tuberculosis* (H37 Ra strain)**
RIBO-prep	PBS, sputum, BAL	$5x10^2$
	Urine	$1x10^3$
	Washing fluids from environmental objects*	$2.5x10^2$ copies/ml
DNA-sorb-B	PBS, sputum	$5x10^2$
	BAL, urine	$1x10^3$
	Washing fluids from environmental objects	$2.5x10^2$ copies/ml

* Analysis can be performed without DNA extraction if washing fluids from environmental objects are added immediately to the reaction mixture for carrying out PCR analysis

13.2 Specificity

The analytical specificity of **eSens MTC QL PCR kit** is ensured by selection of specific primers and probes as well as strict reaction conditions. The primers and probes were checked for possible homologies to all sequences published in gene banks by sequence comparison analysis. The analytical specificity of **eSens MTC QL PCR kit**, which was found to be 100%, was checked by testing 67 reference strains and clinical isolates:

- 16 bacteria representative of the *mycobacterium tuberculosis complex* (*M.tuberculosis, M.bovis, M.bovis BCG*, etc.);
- 23 nontuberculosis mycobacteria (*M.avium, M.fortuitum, M.gordonae, M.intracellulare, M.kansasii, M.marinum, M.paratuberculosis, M.phlei, M.scrofulaceum, M.xenopi, M.smegmatis, M.ulcerans, M.terrae, etc.*);
- Bacteria of other groups (*Brucella abortus*, *B.melitensis, B.ovis, and B.suis; Campylobacter jejuni; Chlamydia suis; Chlamydophila abortus and Ch.felis; Cryptococuss neoformans; Enterobacter cloaca* and *E.faecalis; Enterococcus faecalis; Escherichia coli*; *Klebsiella pneumoniae; Listeria monocytogenes; Moraxella catarrhalis; Neisseria cinerea, N.elongata, N.flava, N.gonor, N.mucosa, N.sicca*, and *N.subflava*; *Pantoea agglomerans; Pasteurella tularensis; Proteus vulgaris* and *P.mirabilis; Pseudomonas aeruginosa*; *Salmonella enteritidis* and *S.typhi; Shigella flexneri* and *Sh.sonne; Staphylococcus aureus;* different clinical isolates of

S.aureus MRSA, *S.faecalis, S.saprophyticus*; and different clinical isolates of *Streptococcus* A, B, C, G, S.*oralis,* and *S.pneumonia*).

The analytical specificity of **eSens MTC QL PCR kit** was estimated by the absence of positive result of the non-tuberculosis bacterium DNA amplification and by the presence of positive result of the *Mycobacterium tuberculosis complex* DNA amplification.

The clinical specificity of **eSens MTC 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		

Ecoli Dx, s.r.o. , Purkyňova 74/2

110 00 Praha 1, Česká republika
Tel: +420 325 209 912

Mobil: +420 739 802 523