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For Professional Use Only

eSens MDR MTC QL PCR kit

REF ES3306B

Instructions for Use

1 INTENDED USE

eSens MDR MTC QL PCR kit is an *in vitro* nucleic acid amplification test for detection of mutations in *Mycobacterium tuberculosis* DNA (*MTC*, *Mycobacterium tuberculosis complex)*, associated with rifampicin (in RRDR region and codon 572 of *rpoB* gene) and isoniazid (in codon 315 of *katG* gene and promoter region of *inhA* gene) resistance, in DNA samples extracted from the biological material (sputum, bronchoalveolar lavage (BAL), bronchial washing fluid, pleural fluid, urine) and *Mycobacterium tuberculosis* cultures using real-time hybridization-fluorescence detection of amplified products. The material for PCR is DNA samples extracted from test material.

The reagent kit can be used only for DNA samples testing with concentration of *MTC* DNA no less than 10^3 genomic equivalents in 1 ml (GE/ml) that was determined by using the reagent kits for quantitative determination of *MTC* DNA manufactured by Federal Budget Institute of Science "Central Research Institute for Epidemiology", or DNA samples with *MTC* DNA detection by using **eSens MDR MTC QL PCR kit**.

Indications and contra-indications for use of the reagent kit

The reagent kit is recommended for use in examination of newly diagnosed patients with tuberculosis (TB) in order to prescribe properly and timely the appropriate TB treatment regimen.

There are no contra-indications with the exception of cases when the material cannot be taken for medical reasons.

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

2 PRINCIPLE OF PCR DETECTION

The principle of testing is based on the simultaneous amplification of *MTC* DNA fragments of the regions of the analyzed mutations associated with rifampicin and isoniazid resistance, and the DNA of the internal control sample (Internal Control-FL (IC)) with real-time hybridization-fluorescence detection. Exogenous internal control (Internal Control-FL (IC)) allows to control all PCR-analysis stages of each individual sample and to identify possible reaction inhibition.

The reaction of DNA fragment amplification with DNA samples obtained at the extraction stage is performed using primers specific to this fragment and polymerase (TaqF) enzyme. 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 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.

Oligonucleotide probes complementary to wild-type *MTC* DNA are used for detection of mutations (except for the probe which detects S531L mutation). Such an approach allows detecting the maximum spectrum of targeted mutations, primarily in RRDR region of *rpoB* gene. When using this approach, the absence of fluorescence increase (absence of *Ct* values) indicates the mutation presence at the hybridization fragment of the probe, complementary to wild-type *MTC* DNA. The used oligonucleotide probes cover the entire RRDR region of *rpoB* gene, region of codon 572 of *rpoB* gene, region of codon 315 of *katG* gene and fragment of promoter region of *inhA* gene (the region containing the -8 and -15 positions). The additional oligonucleotide probe is used. It is complementary to the *rpoB* gene fragment which contains S531L mutation dominant in prevalence (this additional probe is included in the PCR-mix-FL *MTC*-RIF №1, its signal is registered in the channel for the ROX fluorophore).

eSens MDR MTC QL PCR kit uses "hot-start", which greatly reduces the frequency of nonspecifically primed reactions. "Hot-start" is guaranteed by using chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

eSens MDR MTC QL PCR kit contains the system for prevention of contamination by amplicons using the enzyme uracil-DNA-glycosylase (UDG) and deoxyuridine triphosphate (dUTP). The enzyme UDG recognizes and catalyzes the destruction of the DNA containing deoxyuridine, but has no effect on DNA containing deoxythymidine. Deoxyuridine is absent in the authentic DNA, but is always present in amplicons, because dUTP is a part of dNTP mixture in the reagents for the amplification. Due to the deoxyuridine containing contaminating amplicons are sensitive to the destruction by UDG before the DNA-target amplification. So the amplicons cannot be amplified.

The enzyme UDG is thermolabile. It is inactivated by heating at temperature above 50 °C. Therefore, UDG does not destroy the target amplicons which are accumulated during PCR.
The results of amplification are registered in the following fluorescence channels:

Table 1

Channel for fluorophore	**FAM**	**JOE**	**ROX**	**Cy5**
Name of PCR-mix	**DNA-target**			
MTC-RIF №1	RRDR region of *rpoB* gene (wild type)		S531L mutation in RRDR region of *rpoB* gene	RRDR region of *rpoB* gene (wild type)
MTC-RIF №2	RRDR region of *rpoB* gene (wild type)			
MTC-INH №3	fragment of *katG* gene (region of codon 315) (wild type)	fragment of *rpoB* gene out of RRDR (region of codon 572) (wild type)	Internal Control (IC) DNA (artificially synthesized sequence)	fragment of promoter region of *inhA* gene (wild type)

3 CONTENT

eSens MDR MTC QL PCR kit (ES3306B) includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-FL *MTC*-RIF №1	clear liquid from colorless to light lilac colour	0.6	1 tube
PCR-mix-FL *MTC*-RIF №2	clear liquid from colorless to light lilac colour	0.6	1 tube
PCR-mix-FL *MTC*-INH №3	clear liquid from colorless to light lilac colour	0.6	1 tube
PCR-buffer-C	colorless clear liquid	0.3	3 tubes
Polymerase (TaqF)	colorless clear liquid	0.03	3 tubes
C+ *MTC*-wt	colorless clear liquid	0.2	2 tubes
C+ *MTC*-mut	colorless clear liquid	0.2	2 tubes
TE-buffer	colorless clear liquid	0.2	2 tubes

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

eSens MDR MTC QL excel (version 1.0) for automated data processing and Operator manual.

4 ADDITIONAL REQUIREMENTS

- Disposable powder-free gloves and a laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol filters (up to 10, 100, 200, 1000 µl).
- Tube racks.
- Vortex mixer.
- 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) tightly closed 1.5 and 5-ml tubes for pretreatment
  b) thin-walled 0.2-ml PCR tubes with optical transparent domed or flat caps or strips of eight 0.2-ml tubes with optical transparent caps if a plate-type instrument is used;
  c) thin-walled 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 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 the PCR kit if the internal packaging was damaged or its appearance was changed.
- Do not use the PCR kit if the transportation and storage conditions according to the Instruction Manual were not observed.
- 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.
- While observing the conditions of transportation, operation and storage, there are no risks of explosion and ignition.
- Safety Data Sheets (SDS) are available on request.
- The PCR kit is intended for single use for PCR analysis of specified number of samples (see the section “Content”).
- The PCR kit is ready for use in accordance with the Instruction Manual. Use the PCR kit strictly for intended purpose.
- 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

The material for PCR is DNA samples obtained at the extraction stage from the biological material and containing *MTC* DNA no less than 10^3 genomic equivalents in 1 ml (GE/ml) or *MTC* DNA detected with **eSens MDR MTC QL PCR kit**.
The *MTC* DNA samples can be stored before the PCR analysis:

- at the temperature from 2 to 8 °C – for 1 week;
- at the temperature from minus 24 to minus 16 °C – for 1 year.

Transportation of the *MTC* DNA samples is allowed at the temperature from 2 to 8 °C for 1 day.

NOTE: The volume of extracted *MTC* DNA sample must be no less than **30 µl**.

Interfering substances and limitations of using test material samples

In order to control the reaction amplification efficiency the simultaneous amplification of the *MTC* DNA and the Internal Control is used in the PCR kit. The Internal Control is added in each biological sample

at the extraction stage. The presence of internal control signal after the amplification testifies the effectiveness of nucleic acid extraction and the absence of PCR inhibitors.

7 WORKING CONDITIONS

eSens MDR MTC QL PCR kit should be used at the temperature from 20 to 28 °C and relative humidity from 15 to 75 %.

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 the automatic extraction

- **ePure TB DNA Extraction Kit** (E2008).

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.

DNA sample selection suitable for analysis can be performed using **eSens MDR MTC QL excel** (version 1.0).

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

1. Calculate the required quantity of each reagent for the preparation of three reaction mixtures. For one reaction:

   10 µl of **PCR-mix-FL *MTC*-RIF №1** or **PCR-mix-FL *MTC*-RIF №2** or **PCR-mix-FL *MTC*-INH №3,**
   5 µl of **PCR-buffer-C,**
   0.5 µl of **polymerase (TaqF)**.

   Prepare the reaction mixture for the total number of test and control samples plus several extra reactions. See the number of control samples in item 7.

   NOTE: Prepare the reaction mixture just before use.

2. Vortex the tubes with **PCR-mixes-FL, PCR-buffer-C, polymerase (TaqF)** reagents and sediment the drops by short centrifugation.
3. In the new three tubes prepare three reaction n mixtures. Mix the required quantities of **PCR-mix-FL *MTC*-RIF №1** or **PCR-mix-FL *MTC*-RIF №2** or **PCR-mix-FL *MTC*-INH №3, PCR-buffer-C, polymerase (TaqF)**. Sediment the drops by short centrifugation.

4. Take the required (threefold) number of the tubes or strips for DNA amplification of test samples and control samples, put in three rows.
5. Transfer one of the three prepared reaction mixtures of **15 µl** per sample to each row of tubes. Discard the unused reaction mixture.
6. Add **10 µl** of **DNA samples** obtained from test samples at the extraction stage to the three tubes with different reaction mixtures.

NOTE: The volume of extracted *MTC* DNA sample must be no less than **30 µl**.

7. Carry out the control amplification reactions:

C+ wt	–	Add **10 µl** of **C+ *MTC*-wt** to the three tubes with different reaction mixtures labeled C+ wt (Positive Control of Amplification)
C+ mut	–	Add **10 µl** of **C+ *MTC*-mut** to the three tubes with different reaction mixtures labeled C+ mut (Positive Control of Amplification)
NCA	–	Add **10 µl** of **TE-buffer** to the three tubes with different reaction mixtures labeled NCA (Negative Control of Amplification)
C–	–	Add **10 µl** of **the sample extracted from the Negative Control (C–) reagent** to the three tubes with different reaction mixtures labeled C– (Negative control of Extraction).

8.2.2 Amplification

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

Table 2

Amplification and detection 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**	**Fluorescent signal detection**	**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**	**FAM, JOE, ROX, Cy5**	
	72	**15 s**	**—**	

NOTE: This program (table 2) can be used for all the complex of "eSens" reagent kits, which are intended for TB diagnostics. If other tests are carried out simultaneously, the detection is enabled in other used channels.

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 before placing them into the instrument. Insert empty tubes at the edges of reaction module in case of incomplete filling of plate-type instrument.

4. Run the amplification program with fluorescence detection.
5. Analyze results after the amplification program is completed.

8.3 Instrument Settings

eSens MDR MTC QL excel (version 1.0).

Eligibility criteria of *MTC* DNA samples for analysis with **eSens MDR MTC QL PCR kit** by the results obtained with **eSens MDR MTC QL PCR kit**:

The *Ct* value in the channel for the FAM fluorophore for the sample is not greater that the *Ct* values for $C+_{MTC/STI}$ (Positive Control DNA *MTC* /STI) by more than 0.5 cycle.

The following notations are used:

PCR-mix №1 means PCR-mix-FL *MTC*-RIF №1,
PCR-mix №2 means PCR-mix-FL *MTC*-RIF №2,
PCR-mix №3 means PCR-mix-FL *MTC*-INH №3.

Test settings for rotor-type instruments

Channel	Calibrate/Gain Optimisation	Threshold	Dynamic tube	Slope Correct	More Settings/ Outlier Removal
For PCR-mix №1 / PCR-mix №3					
FAM/Green JOE/Yellow ROX/Orange Cy5/Red	from 2 to 5 FI	0.1	On	On	15 %
For PCR-mix №2					
FAM/Green JOE/Yellow ROX/Orange	from 2 to 5 FI	0.1	On	On	10 %
Cy5/Red	from 2 to 5 FI	0.1	On	On	10-30* %

* Recommended values are 10-15 % for PCR-mix-FL *MTC*-RIF №2.

Test settings for plate-type instruments

Channel	Threshold
FAM, HEX ROX, Cy5	Set the threshold line at the level corresponding to 10-20 % of maximum fluorescence level obtained for "C+ wt" sample (in all channels for all three PCR-mixes, except for the ROX channel for PCR-mix №1) and "C+ mut" sample (in the ROX channel for PCR-mix №1) at the last amplification cycle. Make sure that the fluorescence graphs for "C+ wt" and "C+ mut" samples have the typical exponential growth of fluorescent signal.

Note: Set the heating/cooling rate ***Ramp Rate* 2.5 °C/s** by clicking the ***Step Options*** button for each step of cycling.

9 DATA ANALYSIS

NOTE: The following notations are used for this section:

PCR-mix №1 means PCR-mix-FL *MTC*-RIF №1;
PCR-mix №2 means PCR-mix-FL *MTC*-RIF №2;
PCR-mix №3 means PCR-mix-FL *MTC*-INH №3.

Data analysis is performed by the software of the real-time PCR instrument used by measuring fluorescence signal accumulation in four channels:

Table 3

Channel for the fluorophore	**FAM**	**JOE**	**ROX**	**Cy5**
Name of PCR-mix	Signal registration, indicating the amplification product accumulation			
PCR-mix №1	RRDR region of *rpoB* gene (wild type)		S531L mutation in RRDR region of *rpoB* gene	RRDR region of *rpoB* gene (wild type)
PCR-mix №2	RRDR region of *rpoB* gene (wild type)			
PCR-mix №3	fragment of *katG* gene (wild type)	fragment of *rpoB* gene out of RRDR (wild type)	Internal Control (IC) DNA	fragment of promoter region of *inhA* gene (wild type)

Results are interpreted by the crossing (or not-crossing) 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.

NOTE: Data analysis is performed using the under mentioned algorithm or the **eSens MDR MTC QL excel** (version 1.0). Working procedure for the **eSens MDR MTC QL excel** (version 1.0) is described in the Operator manual.

Principle of interpretation is the following:

1. Rifampicin resistance (see point 3 for limitations).

   a) **Rifampicin resistance associated mutations are not detected** if the following three conditions are met for this sample:
      - the *Ct* values are determined in the channels for the FAM, JOE, Cy5 fluorophores and the *Ct* value is absent in the channel for the ROX fluorophore in the tube with PCR-mix №1,
      - the *Ct* values are determined in the channels for the FAM, JOE, ROX, Cy5 fluorophores in the tube with PCR-mix №2,
      - the *Ct* value is determined in the channel for the JOE fluorophore in the tube with PCR-mix №3.

   b) **Rifampicin resistance associated mutations are detected** if at least one of the following conditions is met for this sample:
      - the *Ct* value is absent in one or several channels for the FAM, JOE, Cy5 fluorophores and/or the *Ct* value is determined in the channel for the ROX fluorophore in the tube with PCR-mix №1,
      - the *Ct* value is absent in one or several channels for the FAM, JOE, ROX, Cy5 fluorophores in the tube with PCR-mix №2,

- the *Ct* value is absent in the channel for the JOE fluorophore in the tube with PCR-mix №3.

NOTE: The *Ct* absence is acceptable no more than in four channels listed above for all the three PCR-mixes (see point 3 for limitations), while the available *Ct* values can be greater than the boundary values no more than in three channels, and the *Ct* value in the channel for the ROX fluorophore (Internal Control detection) must be less than the boundary value in a tube with PCR-mix №3.

2. Isoniazid resistance (see point 3 for limitations).

   a) **Isoniazid resistance associated mutations are not detected** for this sample if the *Ct* values are determined in the channels for the FAM and Cy5 fluorophores in the tube with PCR-mix №3.
   b) **Isoniazid resistance associated mutations are detected** for this sample if the *Ct* value is absent in the channel for the FAM (or FAM and Cy5) fluorophore in the tube with PCR-mix №3.
   c) **Low-level isoniazid resistance associated mutations are detected** for this sample if the *Ct* value is absent in the channel for the Cy5 fluorophore and the *Ct* value is determined in the channel for the FAM fluorophore in the tube with PCR-mix №3.

3. Limitations. The listed above results of the detection of mutations, associated with rifampicin and isoniazid resistance, are taken into account if the data obtained for samples do not correspond to the described below options: "***MTC* DNA quantity is not sufficient for the assay**", "**Result is Invalid**" or "**Error**".

   a) ***MTC* DNA quantity is not sufficient for the assay** if one of the following variants is present:
      - the *Ct* values are absent simultaneously in five or more listed below channels: FAM, JOE and Cy5 in the tube with PCR-mix №1, FAM; JOE, ROX and Cy5 in the tube with PCR-mix №2; JOE in the tube with PCR-mix №3; and the *Ct* value determined in the channel for the ROX fluorophore is less than or equal to the boundary value in the tube with PCR-mix №3 (Internal Control detection);
      - the *Ct* values are absent in one or several channels and the *Ct* values are greater than the boundary values in four from the listed below channels: FAM, JOE and Cy5 in the tube with PCR-mix №1; FAM, JOE, ROX and Cy5 in the tube with PCR-mix №2; JOE in the tube with PCR-mix №3; and the *Ct* value determined in the channel for the ROX fluorophore is less than or equal to the boundary value in the tube with PCR-mix №3 (Internal Control detection);
      - the *Ct* values are absent simultaneously in all four channels in the tube with PCR-mix №1 and/or with PCR-mix №2, the present *Ct* values in all the channels in the other tubes are greater than the boundary values except for the *Ct* value determined in the channel for the ROX fluorophore that is less than or equal to the boundary value in the tube with PCR-mix №3 (Internal Control detection).

   DNA sample cannot be analyzed by this reagent kit if the result "***MTC* DNA quantity is not sufficient for the assay**" is obtained, because the content of *MTC* DNA in the sample is less than the analytical sensitivity of the reagent kit or *MTC* DNA is absent.

   b) **Result is invalid** if one of the following variants is present for this sample:

      - the *Ct* value is absent or greater than the boundary value in the channel for the ROX fluorophore in the tube with PCR-mix №3 (Internal Control detection), and the *Ct* values are absent simultaneously in five or more listed below channels: FAM, JOE and Cy5 in the

tube with PCR-mix №1; FAM, JOE, ROX and Cy5 in the tube with PCR-mix №2; JOE in the tube with PCR-mix №3;

- the *Ct* value is absent or greater than the boundary value in the channel for the ROX fluorophore in the tube with PCR-mix №3 (Internal Control detection), and the *Ct* values are absent in one or several other channels, and the *Ct* values are greater than the boundary values in four channels listed below: FAM, JOE and Cy5 in the tube with PCR-mix №1; FAM, JOE, ROX and Cy5 in the tube with PCR-mix №2; JOE in the tube with PCR-mix №3.
- If the result "**Result is invalid**" is obtained the PCR analysis (beginning with the DNA extraction stage) should be repeated for this sample.

c) **Error** if the *Ct* values are absent for the test sample simultaneously in all four channels in one or several tubes with PCR-mixes, whereas one of the following variants is present:

- the present *Ct* values are not greater than the boundary values (all or some of the present *Ct* values);
- the *Ct* value is absent or greater than the boundary value in the channel for the ROX fluorophore in the tube with PCR-mix №3 (Internal Control detection).

If the **"Error"** result is obtained the PCR analysis (beginning with the DNA extraction stage) should be repeated for this sample.

The result of the PCR analysis is considered reliable only if the results obtained for controls of extraction stages and DNA amplification are correct (according to Table 4 and Table 5).

Table 4

Results for controls

Name of PCR-mix	Control	Stage for control	*Ct* value in the channel for fluorophore			
			FAM	JOE	ROX	Cy5
PCR-mix №1	NCA	PCR	Absent	Absent	Absent	Absent
PCR-mix №2			Absent	Absent	Absent	Absent
PCR-mix №3			Absent	Absent	Absent	Absent
PCR-mix №1	C+ wt	PCR	< boundary value	< boundary value	Absent	< boundary value
PCR-mix №2			< boundary value	< boundary value	< boundary value	< boundary value
PCR-mix №3			< boundary value	< boundary value	< boundary value	< boundary value
PCR-mix №1	C+ mut	PCR	Absent	Absent	< boundary value	Absent
PCR-mix №2			< boundary value	Absent	< boundary value	Not taken into account
PCR-mix №3			Absent	Absent	< boundary value	Absent
PCR-mix №1	C–	DNA extraction and PCR	Absent	Absent	Absent	Absent
PCR-mix №2			Absent	Absent	Absent	Absent
PCR-mix №3			Absent	Absent	< boundary value	Absent

Table 5

Boundary *Ct* values

Sample	Rotor-type Instruments				Plate-type Instruments			
	Channel for fluorophore							
	FAM	JOE	ROX	Cy5	FAM	JOE	ROX	Cy5
PCR-mix №1								
C+wt	28	29	-	30	31	32	-	33
C+ mut	-	-	29	-	-	-	31	-
NCA	-	-	-	-	-	-	-	-
C-	-	-	-	-	-	-	-	-
Test samples	30.5	30.5	40	31	33.5	33.5	40	33.5
PCR-mix №2								
C+wt	31	30.5	30	32	33.5	33.5	33	34
C+ mut	40	-	31	Not taken into account	40	-	34	Not taken into account
NCA	-	-	-	-	-	-	-	-
C-	-	-	-	-	-	-	-	-
Test samples	33	31	32	33.5	34	33.5	33.5	34.5
PCR-mix №3								
C+wt	28	28	26	31	32	32	28	34
C+ mut	-	-	26	-	-	-	28	-
NGA	-	-	-	-	-	-	-	-
C-	-	-	27	-	-	-	29	-
Test samples	37	31	27	37	39	34.5	29	39

10 TROUBLESHOOTING

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

1. If the *Ct* value determined for the Positive Control of amplification (C+ wt) is absent or greater than the boundary *Ct* value in the channels where it must be “less than the boundary *Ct* value”, according to Table 4. The PCR analysis should be repeated for all samples.
2. If the *Ct* value determined for the Positive Control of amplification (C+ mut) is absent or greater than the boundary *Ct* value in the channels where it must be “less than the boundary *Ct* value”, according to Table 4, or the *Ct* value is determined in the channels for fluorophores where it must be “absent”, according to Table 4. The PCR analysis should be repeated for all samples.
3. If the *Ct* value is determined for the Negative Control of amplification (NCA) in the one or several channels. The contamination of laboratory with amplification fragments or contamination of reagents, test samples is probable at any stage of PCR analysis. Measures for detecting and elimination of contamination source must be taken. The PCR analysis should be repeated for all samples.
4. If the *Ct* value is determined for the Negative Control of Extraction (C–) in the one or several channels on which it must be “absent”, according to Table 4. The contamination of laboratory with amplification fragments or contamination of reagents, test samples is probable at any stage of PCR analysis. Measures for detecting and elimination of contamination source must be taken. The PCR analysis (beginning with the DNA extraction stage) should be repeated for all samples.
5. If the *Ct* value is determined for the test sample, whereas the area of typical exponential growth of fluorescence is absent (the graphic looks like approximate straight line). It is necessary to

check that threshold line or parameters of threshold line measurement are correct. If the result has been obtained with the correct threshold line level, the amplification and detection should be repeated for this sample.

11 TRANSPORTATION

eSens MDR 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 MDR MTC QL PCR kit** are to be stored at 2–8 °C when not in use (except for PCR-buffer-C and polymerase (TaqF)). All components of the **eSens MDR MTC 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-buffer-C and polymerase (TaqF) are to be stored at the temperature from minus 24 to minus 16 °C.

NOTE: PCR-mix-FL *MTC*-RIF №1, PCR-mix-FL *MTC*-RIF №2 and PCR-mix-FL *MTC*-INH №3 are to be kept away from light.

13 SPECIFICATIONS

13.1 Analytical sensitivity (limit of detection)

Table 6

Test material	PCR kit	Analytical sensitivity (limit of detection), GE/ml
DNA sample obtained from the biological material at the extraction stage	ES3306B	$1x10^3$

13.2 Analytical specificity

The analytical specificity of **eSens MDR MTC 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 MDR MTC QL PCR kit** was confirmed by DNA testing of the following microbial strains: *Corynebacterium jeikeium*, *Corynebacterium xerosis*, *Corynebacterium minutissimum*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *E.coli*, *Neisseria meningitidis*, *Haemophilus influenzae*, *Stenotrophomonas maltophilia*, *Pseudomonas stutzeri*, *Pseudomonas putida*, *Pseudomonas aeruginosa*, *Pseudomonas koreensis*, *Enterococcus faecium*, clinical isolates DNA of the following nontuberculous mycobacteria: *M. avium* complex, *M. kansasii*, *M. fortuitum* group, and also genomic DNA of human.

The clinical specificity of **eSens MDR MTC QL PCR kit** was confirmed in laboratory clinical trials.

13.3 Diagnostic characteristics

Diagnostic criteria of the reagent kit was assessed by comparing the study results of 87 samples of different types of biomaterial obtained from patients with pulmonary tuberculosis and other

tuberculosis localization with the data of phenotypic determination of *MTC* drug sensitivity to rifampicin and isoniazid. The absolute concentration method on Lowenstein-Jensen solid medium was used as a reference assay for determination of *MTC* drug sensitivity.

Table 7

The results of testing eSens MDR MTC QL PCR kit in comparison with the reference assay

Samples type	TB drugs	Number of test samples	The results of application of eSens MDR MTC QL PCR kit	Results of using the reference assay	
				MTC resistance to TB drugs (positive)	*MTC* sensitivity to TB drugs (negative)
DNA samples extracted from the biological material	Rifampicin	87	Mutations were detected (positive)	41	2
			Mutations were not detected (negative)	0	44
	Isoniazid	87	Mutations were detected (positive)	46	2
			Mutations were not detected (negative)	0	39

Table 8

Diagnostic characteristics of eSens MDR MTC QL PCR kit

Samples type	TB drugs	Diagnostic sensitivity*, (with a confidence level of 90 %) no less than %	Diagnostic specificity**, (with a confidence level of 90 %) no less than %
DNA samples extracted from the biological material	Rifampicin	94.5	89.0
	Isoniazid	95.0	87.5

***** Relative sensitivity in comparison with applied reference method.

****** Relative specificity in comparison with applied reference method.

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