

AmpliSens® MTC-MDR-FRT PCR kit



For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

	Catalogue number		Contains sufficient for <n> tests
	Batch code		Use-by Date
	In vitro diagnostic medical device		Consult instructions for use
	Version		Keep away from sunlight
	Temperature limit		Keep dry
	Manufacturer		Negative control of amplification
	Date of manufacture		Negative control of extraction
	Authorized representative in the European Community		Positive controls of amplification
	Caution		Internal Control

1. INTENDED USE

AmpliSens® MTC-MDR-FRT 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 10³ 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 AmpliSens® MTC-FRT PCR kit with the boundary *Ct* values meeting the criteria specified in the *Important Product Information Bulletin* enclosed to the 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).

AmpliSens® MTC-MDR-FRT 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.

The PCR kit variant FRT-50 F 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 <i>rpoB</i> gene (wild type)		S531L mutation in RRDR region of <i>rpoB</i> gene	RRDR region of <i>rpoB</i> gene (wild type)
MTC-RIF №2	RRDR region of <i>rpoB</i> gene (wild type)			
MTC-INH №3	fragment of <i>katG</i> gene (region of codon 315) (wild type)	fragment of <i>rpoB</i> gene out of RRDR (region of codon 572) (wild type)	Internal Control (IC) DNA (artificially synthesized sequence)	fragment of promoter region of <i>inhA</i> gene (wild type)

3. CONTENT

AmpliSens® MTC-MDR-FRT PCR kit is produced in 2 forms:

variant FRT-50 F, H-3611-1-CE

variant FRT-L, H-3612-1-4-CE

Variant FRT-50 F 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

Variant FRT-50 F is intended for 55 reactions (including controls).

Variant FRT-L includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix MTC-RIF №1-Lyo	white powder	–	48 tubes of 0.2 ml
PCR-mix MTC-RIF №2-Lyo	white powder	–	48 tubes of 0.2 ml
PCR-mix MTC-INH №3-Lyo	white powder	–	48 tubes of 0.2 ml
C+ MTC-wt	colorless clear liquid	0.5	2 tubes
C+ MTC-mut	colorless clear liquid	0.5	2 tubes
TE-buffer	colorless clear liquid	0.5	2 tubes

Variant FRT-L is intended for 48 reactions (including controls).

AmpliSens® MTC-MDR software (version 1.0) in Microsoft® Excel format 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 (Bio-Rad, USA)).
- Disposable polypropylene PCR tubes:
 - tightly closed 1.5 and 5-ml tubes for pretreatment
 - 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 for PCR kit variant FRT-50 F;
 - 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 for variant FRT-50 F.
- 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 distinctly 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 AmpliSens® *MTC-FRT* PCR kit, with the *Ct* value meeting the criteria specified in the *Important Product Information Bulletin* enclosed to this 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 for **Variant FRT-50 F** must be no less than **30 µl**. The volume of extracted *MTC* DNA sample for **Variant FRT-L** must be no less than **75 µl**.

Interfering substances and limitations of using test material samples

The *MTC* DNA samples are not applicable for analysis if concentration, volume, storage conditions/stability and transportation do not conform the requirements specified in the section "*Sampling and handling*".

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

AmpliSens® *MTC-MDR-FRT* PCR kit PCR kit should be used at the temperature from 20 to 28 °C and relative humidity from 15 to 75 %.

8. PROTOCOL

8.1. Preparing PCR

8.1.1 Preparing tubes for PCR

DNA sample selection suitable for analysis in accordance with requirements of "*Sampling and handling*" section can be performed using AmpliSens® *MTC-MDR* software.

Variant FRT-50 F

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

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.

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 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**.

Avoid transferring of sorbent together with the DNA samples extracted by reagent kit with method of sorption on silica gel or magnetic separation method.

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).

Variant FRT-L

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

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

1. Take the required number of the tubes with three ready-to-use lyophilized reaction mixtures **PCR-mixes *MTC-RIF* №1-Lyo**, ***MTC-RIF* №2-Lyo** and ***MTC-INH* №3-Lyo** for amplification of DNA from test and control samples (the number of control samples see in point 3).

2. Add **25 µl** of **DNA samples** extracted from test samples into the prepared three tubes with different reaction mixtures.

NOTE: The volume of extracted *MTC* DNA sample must be no less than **75 µl**.

Avoid transferring of sorbent together with the DNA samples extracted by reagent kit with method of sorption on silica gel or magnetic separation method.

3. Carry out the control reactions:

C+ wt – Add **25 µl** of **C+ *MTC-wt*** to the three tubes with different reaction mixtures labeled C+ wt (Positive Control of Amplification)

C+ mut – Add **25 µl** of **C+ *MTC-mut*** to the three tubes with different reaction mixtures labeled C+ mut (Positive Control of Amplification)

NCA – Add **25 µl** of **TE-buffer** to the three tubes with different reaction mixtures labeled NCA (Negative Control of Amplification)

C– – Add **25 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).

NOTE: Mix the tubes thoroughly by pipetting avoiding foaming.

8.3.2. Amplification

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

Table 2
Amplification and detection program for fluorescent signal "*MTC*" for rotor-type instruments¹ and plate-type instruments²

Step	Temperature, °C	Time	Fluorescent signal detection	Cycles
1	95	15 min	—	1
2	95	15 s	—	5
	65	30 s	—	
3	72	15 s	—	40
	95	15 s	—	
	65	30 s	FAM, JOE, ROX, Cy5	
	72	15 s	—	

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

2. Adjust the fluorescence channel sensitivity according to the *Important Product Information Bulletin*.

3. Insert tubes into the reaction module of the device.

It is recommended to sediment drops from walls of tubes by short centrifugation before placing them into the instrument.

NOTE: 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.

¹ For example, Rotor-Gene (QIAGEN, Germany).

² For example, CFX 96 (Bio-Rad).

9. DATA ANALYSIS

The following notations are used for this section:

NOTE: PCR-mix №1 means PCR-mix-FL *MTC-RIF* №1 or PCR-mix *MTC-RIF* №1-Lyo;
 PCR-mix №2 means PCR-mix-FL *MTC-RIF* №2 or PCR-mix *MTC-RIF* №2-Lyo;
 PCR-mix №3 means PCR-mix-FL *MTC-INH* №3 or PCR-mix *MTC-INH* №3-Lyo.

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 <i>rpoB</i> gene (wild type)	S531L mutation in RRDR region of <i>rpoB</i> gene	RRDR region of <i>rpoB</i> gene (wild type)	
PCR-mix №2	RRDR region of <i>rpoB</i> gene (wild type)			
PCR-mix №3	fragment of <i>katG</i> gene (wild type)	fragment of <i>rpoB</i> gene out of RRDR (wild type)	Internal Control (IC) DNA	fragment of promoter region of <i>inhA</i> 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 AmpliSens® *MTC-MDR* software version 1.0. Working procedure for the AmpliSens® *MTC-MDR* software 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.

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.

NOTE:

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.

NOTE: Boundary Ct values are specified in the *Important Product Information Bulletin* enclosed to the PCR kit.

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 the *Important Product Information Bulletin* enclosed to the PCR kit).

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			Absent	Absent	Absent	Absent
PCR-mix №2	NCA	PCR	Absent	Absent	Absent	Absent
PCR-mix №3			Absent	Absent	Absent	Absent
PCR-mix №1			< boundary value	< boundary value	Absent	< boundary value
PCR-mix №2	C+ wt	PCR	< boundary value	< boundary value	< boundary value	< boundary value
PCR-mix №3			< boundary value	< boundary value	< boundary value	< boundary value
PCR-mix №1			Absent	Absent	< boundary value	Absent
PCR-mix №2	C+ mut	PCR	< boundary value	Absent	< boundary value	Not taken into account
PCR-mix №3			Absent	Absent	< boundary value	Absent
PCR-mix №1			Absent	Absent	Absent	Absent
PCR-mix №2	C-	DNA extraction and PCR	Absent	Absent	Absent	Absent
PCR-mix №3			Absent	Absent	< boundary value	Absent

10. TROUBLESHOOTING

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

- 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.
- 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.
- 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.
- 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.
- 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.

If you have any further questions or if you encounter problems, please contact our Authorized representative in the European Community.

11. TRANSPORTATION

AmpliSens® *MTC-MDR-FRT* PCR kit should be transported at 2–8 °C for no longer than 5 days.

12. STABILITY AND STORAGE

All components of the AmpliSens® *MTC-MDR-FRT* 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 AmpliSens® *MTC-MDR-FRT* 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

NOTE: PCR-mix *MTC-RIF* №1-Lyo, PCR-mix *MTC-RIF* №2-Lyo and PCR-mix *MTC-INH* №3-Lyo are to be kept in packages with a desiccant away from light

13. SPECIFICATIONS

13.1. Analytical sensitivity (limit of detection)

Table 5

Test material	PCR kit	Analytical sensitivity (limit of detection), GE/ml
DNA sample obtained from the biological material at the extraction stage	variant FRT-50 F, variant FRT-L	1x10 ³

The claimed features are achieved while respecting the rules specified in the section "Sampling and Handling".

13.2. Analytical specificity

The analytical specificity of **AmpliSens[®] MTC-MDR-FRT** 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 **AmpliSens[®] MTC-MDR-FRT** 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 **AmpliSens[®] MTC-MDR-FRT** 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 6

The results of testing **AmpliSens[®] MTC-MDR-FRT** PCR kit in comparison with the reference assay

Samples type	TB drugs	Number of test samples	The results of application of AmpliSens[®] MTC-MDR-FRT PCR kit	Results of using the reference assay	
				<i>MTC</i> resistance to TB drugs (positive)	<i>MTC</i> 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 7

Diagnostic characteristics of **AmpliSens[®] MTC-MDR-FRT** PCR kit

Samples type	TB drugs	Diagnostic sensitivity ³ , (with a confidence level of 90 %) no less than %	Diagnostic specificity ⁴ , (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

14. REFERENCES

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2. Campbell EA, Korzheva N, Mustaev A. et al. Structural Mechanism for Rifampicin Inhibition of Bacterial RNA Polymerase // Cell, 2001, Mar 23;104(6):901-12.
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4. Kostera J, Leckie G, Tang N et al. Analytical and clinical performance characteristics of the Abbott RealTime MTB RIF/INH Resistance, an assay for the detection of rifampicin and isoniazid resistant *Mycobacterium tuberculosis* in pulmonary specimens // Tuberculosis (Edinb) 2016 Dec; 101: 137-143.

15. QUALITY CONTROL

In compliance with Federal Budget Institute of Science "Central Research Institute for Epidemiology" ISO 13485-Certified Quality Management System, each lot of the **AmpliSens[®] MTC-MDR-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
02.06.20 MM	Footer	The phrase "Not for use in the Russian Federation" was added
16.03.21 MA	—	The name, address and contact information for Authorized representative in the European Community was changed

AmpliSens[®]



Ecoli Dx, s.r.o., Purkyňova 74/2
110 00 Praha 1, Czech Republic
Tel.: +420 325 209 912
Cell: +420 739 802 523



Federal Budget Institute of Science "Central Research Institute for Epidemiology"
3A Novogireevskaya Street
Moscow 111123 Russia

³ Relative sensitivity in comparison with applied reference method.

⁴ Relative specificity in comparison with applied reference method.