ECOLI

eSens

CE

IVD

For Professional Use Only

eSens MDR MBL QL PCR kit

REF ES4301A

Instructions for Use

1 INTENDED USE

eSens MDR MBL QL PCR kit is an *in vitro* nucleic acid amplification test for detection of acquired carbapenems genes of metallo-β-lactamase class, types IMP, VIM, and NDM, in the biological material (DNA extracted from samples of pure bacterial culture, positive blood culture, a mixture of bacterial cultures obtained by primary seeding of clinical material (liquor, bronchoalveolar lavage (BAL), traumatic discharge, etc.) to solid or liquid medium) and in the clinical material (urine, oropharyngeal and rectal swabs) using real-time fluorescence-hybridization detection of amplified products.

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

2 PRINCIPLE OF PCR DETECTION

MDR MBL detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific MDR MBL 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 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.

The DNA extraction from the biological material is carried out in the presence of the Internal Control-FL which allows to control the procedure of examination of each sample. The fluorescent labels are attached to the oligonucleotide probes specific to different DNA-targets. This allows to register the accumulation of specific amplification product of each DNA-target by the detection of the intensity of fluorescent signal through the relevant channel during the PCR with the help of thermo-cycler with the real-time detection of fluorescent signal system.

The PCR kit contains the system for prevention of contamination by amplicons using the enzyme uracil-DNA-glycosylase (UDG) and 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 amplification results of genes fragments of IMP, VIM, and NDM types are registered separately for each type through three different channels, the results of amplification of VIM type is registered through the channel for the FAM fluorophore, the IMP type is registered through the channel for the JOE fluorophore, a NDM-type is registered through the channel for the Cy5 fluorophore. Through the channel for the ROX fluorophore the amplification product of Internal Control is detected.

Table 1

Channel for fluorophore	**FAM**	**JOE**	**ROX**	**Cy5**
DNA target	VIM-type MBL genes	IMP-type MBL genes	Internal Control	NDM-type MBL genes

3 CONTENT

eSens MDR MBL QL PCR kit (ES4301A) includes:

Reagent	**Description**	**Volume, ml**	**Quantity**
PCR-mix-1-FRT MBL	clear liquid from colorless to light lilac colour	1.2	1 tube
PCR-mix-2-FRT	colorless clear liquid	0.3	2 tubes
Polymerase (TaqF)	colorless clear liquid	0.03	2 tubes
TE-buffer	colorless clear liquid	0.2	1 tube
Positive Control-2-MBL	colorless clear liquid	0.2	1 tube
Negative Control (C-)*	colorless clear liquid	1.2	1 tube
Internal Control-FL (IC)**	colorless clear liquid	1.0	1 tube

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

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

eSens MDR MBL QL PCR kit is intended for 110 reactions (including controls).

4 ADDITIONAL REQUIREMENTS

- Disposable screwing or tight-fitting polypropylene tubes (1.5-ml) for a pretreatment of the material.
- DNA extraction kit
- Disposable powder-free gloves and a laboratory coat.
- Pipettes (adjustable).
- Disposable pipette tips with filter (up to 100 µl).

- Tube racks (for 0.1- or 0.2-ml tube).
- 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 at 2 to 8 °C.
- Deep-freezer at 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 filters and use a new tip for every procedure.
- Store extracted positive material (samples, controls and amplicons) away from all other reagents and add it to the reaction mix in a separate area.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats and eye protection when handling specimens and reagents. 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 a biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills using a disinfectant, such as 0.5% sodium hypochlorite or another suitable disinfectant.
- Avoid sample or reagent contact with the skin, eyes, and mucous membranes. If any of these solutions come into contact, rinse the injured area immediately with water and seek medical advice immediately.
- Material Safety Data Sheets (MSDS) 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 Areas. Do not return samples, equipment and reagents to the area in which 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 MDR MBL QL PCR kit is intended for the analysis of DNA fragments of genes of IMP-, VIM-, and NDM-type acquired metallo-β-lactamases with DNA extraction kits from the biological material (DNA extracted from samples of pure bacterial cultures, positive blood cultures, a mixture of bacterial

cultures obtained by primary seeding clinical material (liquor, bronchoalveolar lavage (BAL), traumatic discharge, etc.) to solid or liquid medium) and in the clinical material (urine, oropharyngeal and rectal swabs).

Sampling

6.1 Oropharyngeal and rectal swabs.

Oropharyngeal and rectal swabs should be placed in **Transport Medium with Mucolytic Agent** (952-CE).

6.2 A mixture of bacterial cultures .

A mixture of bacterial cultures obtained by seeding of clinical material to solid medium 10^7-10^9 bacterial cells.

Pretreatment

6.3 Blood cultures.

Blood cultures, a mixture of bacterial cultures obtained by primary seeding clinical material to liquid medium. Transfer from 0.1 to 0.25 ml the blood culture or the seeding to an enrichment medium in a sterile disposable tube 1.5 ml (use a disposable syringe).
Centrifuge the tubes at 10,000 g (12,000 rpm in MiniSpin, Eppendorf) for 10 min. Discard the supernatant by using a vacuum aspirator with trap flask (use new tip without filter for each sample). Making sure that the pellet is not disturbed.

6.4 Urine

Shake the bottle of urine. Transfer 1 ml of the urine in a sterile disposable 1.5 ml tube (use new tip with filter for each sample). Centrifuge the tubes at 10,000 g (12,000 rpm in MiniSpin, Eppendorf) for 10 min. In case if urine contains a lot of salts, resuspended only the upper layer pellet salts in 1 ml, then centrifuged again. Discard the supernatant by using a vacuum aspirator with trap flask (use new tip without filter for each sample). Making sure that the pellet is not disturbed.
Use the pellet for DNA extraction.
Samples (pellets) can be stored at a temperature from minus 24 to minus 16 °C for 1 week and at no more than minus 68 °C for 1 year.

7 WORKING CONDITIONS

eSens MDR MBL 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-AM** (K1-12-100-CE) is recommended to use for DNA extraction from the samples **positive blood cultures, a mixture of bacterial cultures obtained by seeding clinical material to liquid medium**, after pretreatment, the samples **positive blood cultures, a mixture of**

bacterial cultures obtained by seeding clinical material to solid medium according to the Instruction Manual of reagents kit.

- **DNA-sorb-AM** (K1-12-100-CE) and **RIBO-prep** (K2-9-Et-100-CE) are recommended to use for DNA extraction from the samples of **urine** after pretreatment according to the Instruction Manual of reagents kit.
- **DNA-sorb-AM** (K1-12-100-CE) is recommended to use for DNA extraction from the samples **oropharyngeal and rectal swabs** according to the Instruction Manual of reagents kit.

- For the automatic extraction

- **ePure Bacterial DNA Extraction Kit** (E2006)

NOTE: Extract the DNA according to the manufacturer's protocol.
DNA extraction from each of the examined sample is carried out in the presence of **Internal control-FL (IC)**. As a probe C- in-use **Negative Control (C–)** reagent.

NOTE: In case of extraction from samples that resemble pellets after pre-treatment, the Lysis buffer should be added directly to tube with the pellet, using a new filter tip for each sample.

NOTE: In case of extraction from samples of a pure bacterial culture or a mixture of bacterial cultures obtained by seeding of clinical material to solid medium bacterial cells are taken with a sterile loop (or sterile tip) in a quantity 10^7-10^9 cells are placed directly into a 1.5 ml tube containing lysis solution of DNA-sorb-AM kit.

NOTE: It is not recommended to carry out DNA extraction from blood culture samples, a pure culture or mixture of bacterial cultures obtained by seeding to medium and samples of other biological material, simultaneously, because there is a high risk of contamination from positive blood culture or bacterial cultures containing high DNA pathogen concentrations.

8.2 Preparing the 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.

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

NOTE: Prepare the reaction mixture just before use

The reagents are mixed based on one reaction:

10 µl PCR-mix-1-FRT MBL,
5 µl PCR-mix-2–FRT,
0.5 µl of polymerase (TaqF).

1. Prepare the mixture of **PCR-mix-2–FRT** and **polymerase (TaqF)**. Pour all the content of one tube with **polymerase (TaqF) (30 µl)** into the tube with **PCR-mix-2-FRT (300 µl)** and stir it carefully on the vortex not allowing foaming. Label the tube by the date of the preparation of the mix.

NOTE: The prepared mixture is intended for 60 reactions. The mixture is to be stored at 2–8 °C during 3 months and used when it is necessary.

NOTE: If the mix can't be used within three months it is necessary to prepare the mix for less number of reactions – for example mix **150 µl** of **PCR-mix-2-FRT** and **15 µl** of **polymerase (TaqF)** (such mix is intended for 30 reactions).

2. Vortex the tube with **PCR-mix-1-FRT MBL**, then centrifuge briefly.

Calculate the necessary amount of reactions including tests of examined and control samples. It can be done according to the table 2. It should be took into account that even for one test of the examining sample three control reactions should be done: **C+, C– and NCA**. The reagents should be taken with reserve, for example for examining N samples the reagents for (N+1) reactions should be prepared.

Table 2

Scheme of reaction mixture preparation

	Reagent volume for specified number of reactions	
Reagent volume per one reaction, µl	**10.0**	**5.0**
Number of reactions*	**PCR-mix-1-FRT MBL**	**The mixture of PCR-mix-2-FRT and Polymerase (TaqF)**
2	**60**	**30**
3	**70**	**35**
4	**80**	**40**
5	**90**	**45**
6	**100**	**50**
7	**110**	**55**
8	**120**	**60**
9	**130**	**65**
10	**140**	**70**
11	**150**	**75**
12	**160**	**80**
13	**170**	**85**
14	**180**	**90**
15	**190**	**95**
16	**200**	**100**
17	**210**	**105**
18	**220**	**110**
19	**230**	**115**
20	**240**	**120**
21	**250**	**125**
22	**260**	**130**

23	**270**	**135**
24	**280**	**140**
25	**290**	**145**

***** Number of test samples (N) including 1 control of extraction stage, 2 controls of amplification, and 1 extra reaction (N+1+2+1).

3. In a separate tube prepares the reaction mixture. Mix the appropriate amount of **PCR-mix-1-FRT MBL** and the mixture of **PCR-mix-2-FRT** and **polymerase (TaqF)**.
4. Take the required number of tubes or strips for DNA amplification of examined and control samples.
5. Transfer **15 µl** of the prepared mixture to each tube.
6. Add **10 µl** of DNA obtained from the examined and control samples.
7. Carry out the control reactions:

NCA	–	Add **10 µl** of **TE-buffer** to the tube labeled NCA (Negative Control of Amplification)
C+	–	Add **10 µl** of **Positive Control-1-MBL (C+$_1$)** to the tube labeled C+
C–	–	Add **10 µl** of **the sample extracted from the Negative Control (C-) reagent** to the tube labeled C–.

8.2.2 Amplification

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

Table 3

eSens-1 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**	**5 s**	**5**	**95**	**5 s**	**5**
	60	**20 s**		**60**	**20 s**	
	72	**15 s**		**72**	**15 s**	
3	**95**	**5 s**	**40**	**95**	**5 s**	**40**
	60	**20 s Fluorescence acquiring**		**60**	**30 s Fluorescence acquiring**	
	72	**15 s**		**72**	**15 s**	

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

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

3. Run the amplification program with fluorescent signal detection.
4. 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 to 10 FI	0.1	On	On	5-15 %
JOE/Yellow	from 4 to 8 FI	0.1	On	On	5-15 %
ROX/Orange	from 4 to 8 FI	0.1	On	On	5-15 %
Cy5/Red	from 4to 8 FI	0.1	On	On	5-15 %

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, JOE/HEX, C5	Set the threshold line for each channel at the level of 10-20 % of maximum fluorescence obtained for the Positive Control in the last amplification cycle.
ROX	Set the threshold line at the level of 10-20 % of maximum fluorescence obtained for the Negative Control of Extraction (C-).

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 **VIM-type of MBL genes** fragments amplification product is detected in the channel for the **FAM** fluorophore.
- The signal of the **IMP-type of MBL genes** fragments amplification product is detected in the channel for the **JOE** fluorophore.
- The signal of the **Internal Control-FL (IC)** amplification product is detected in the channel for the **ROX** fluorophore.
- The signal of the **NDM-type of MBL genes** fragments amplification product is detected in the channel for the **Cy5** fluorophore.

Results are interpreted by the crossing (or not-crossing) the fluorescence curve with the threshold line set at the level of exponential growth that corresponds to the presence (or absence) of a *Ct* value of the DNA target in the corresponding column of the results grid.
Principle of interpretation is the following:

- **MBL genes** is **detected** if the *Ct* value determined in the results grid in the channel for the FAM or/and JOE, or/and Cy5 fluorophore is less than the boundary *Ct* value. Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.
- **MBL genes** is **not detected** in a sample if the *Ct* value is not determined (absent) in the channels for FAM, JOE, Cy5 fluorophores, whereas the *Ct* value determined in the channel for the ROX fluorophore is less than the boundary *Ct* value.
- The result is **invalid** if the *Ct* value is not determined (absent) in the channel for FAM, JOE, Cy5 fluorophores, whereas the *Ct* value in the channel for the ROX fluorophore is not determined

(absent) or greater than the specified boundary *Ct* value. In such cases, the PCR analysis should be repeated starting from the DNA extraction stage. If the same result is obtained in the second run, re-sampling of material is recommended.

The result of the analysis is considered reliable only if the results obtained for Positive and Negative Controls of amplification as well as for the Negative Control of extraction of DNA are correct (see Table 4 and Table 5).

Table 4

Results for controls

Control	**Stage for control**	***Ct* value in the channel for fluorophore**	
		FAM, JOE, Cy5	**ROX**
C–	DNA extraction	Absent	< boundary value
NCA	PCR	Absent	Absent
C+	PCR	< boundary value	Not evaluated

Table 5

Boundary *Ct* values

Sample	**Rotor-type instruments**				**Plate-type instruments**			
	Channel for fluorophore							
	FAM	**JOE**	**ROX**	**Cy5**	**FAM**	**JOE**	**ROX**	**Cy5**
Positive Control (C+)	<31	<31	-	<33	<34	<34	-	<34
Negative Control (C-)	-	-	<31	-	-	-	<34	-
Bacterial cultures samples*	<30	<30	<31	<31	<31	<31	<34	<32
Urine and swabs samples	-	-	<31	-	-	-	<34	-

* Only for samples of positive blood cultures, a mixture of bacterial cultures obtained by seeding of the clinical material, a pure culture.

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+) in the channels for the FAM, JOE, Cy5 fluorophores is greater than the boundary *Ct* value or absent, the amplification and detection should be repeated for all samples.
2. If for the Negative Control of DNA extraction (C-) and/or Negative Control of amplification (NCA) the value of the threshold cycle (*Ct*) is registered through one of the channels for FAM or/and JOE, or/and Cy5 fluorophores, the PCR analysis should be repeated for all samples in which the *Ct* value was detected in the respective channel.

11 TRANSPORTATION

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

NOTE: PCR-mix-1-FRT MBL is to be kept away from light

13 SPECIFICATIONS

13.1 Analytical sensitivity

Biological material	Transport Medium	Nucleic acid extraction kit	Sensitivity, copies/ml*
Blood cultures, a mixture of bacterial cultures obtained by primary seeding clinical material to solid or liquid medium	—	DNA-sorb-AM	$1x10^5$
Urine	—	DNA-sorb-AM	$5x10^2$
		RIBO-prep	
Oropharyngeal and rectal swabs	Transport Medium for Swabs or Transport Medium with Mucolytic Agent	DNA-sorb-AM	$2x10^3$

***** It is necessary to observe the pretreatment rules and the recommended volume of the test sample for obtain this sensitivity.

The genes of MBL of corresponding types were identified using this reagents kit then the DNA samples of control strains, carrying the genes of known MBL of VIM-1, VIM-2, VIM-4, VIM-10, IMP-1, IMP-2, IMP -12, IMP-13 types, were analyzed.

13.2 Analytical specificity

The analytical specificity of **eSens MDR MBL 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 nonspecific reactions were absent testing the human DNA samples and the following microorganisms' DNA: *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca, Serratia marcescens, Acinetobacter baumannii, Proteus mirabilis, Enterococcus faecalis, Staphylococcus* spp., *Streptococcus* spp., *Candida* spp.

The clinical specificity of **eSens MDR MBL 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
LOT	Batch code
IVD	*In vitro* diagnostic medical device
VER	Version
	Temperature limit
	Manufacturer
	Date of manufacture
EC REP	Authorized representative in the European Community
	Caution
	Contains sufficient for <n> tests
	Use-by Date
	Consult instructions for use
	Keep away from sunlight
NCA	Negative control of amplification
C-	Negative control of extraction
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