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For Professional Use Only

AmpliSens® MDR KPC/OXA-48-FRT

PCR kit

Instruction Manual

AmpliSens®

EC REP

Ecoli s.r.o., Studenohorska 12
841 03 Bratislava 47
Slovak Republic
Tel.: +421 2 6478 9336
Fax: +421 2 6478 9040

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

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1. INTENDED USE

AmpliSens® MDR KPC/OXA-48-FRT PCR kit is an *in vitro* nucleic acid amplification test for detection of genes of KPC-type, OXA-48-like (OXA-48- and OXA-162-type) acquired carbapenemases in the biological material (DNA extracted from samples of a pure bacterial culture, a 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) by using real-time hybridization-fluorescence detection of amplified products.

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

2. PRINCIPLE OF PCR DETECTION

DNA fragments of genes detection of KPC-type, OXA-48-like (OXA-48- and OXA-162-type) acquired carbapenemases by the polymerase chain reaction (PCR) with real-time hybridization-fluorescence detection contains two steps: DNA extraction from the biological material and amplification of the DNA fragments of genes with real-time hybridization-fluorescence detection. The DNA extraction from the biological material is carried out with the presence of the Internal Control-FL, which allows to control the procedure of examination of each sample. Then with the obtained DNA samples the amplification with the help of specific primers and Taq-polymerase enzyme with the simultaneous detection with the help of specific fluorescently labeled oligonucleotide probes is carried out. Fluorescence labels are attached to 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 amplification results of KPC-type, OXA-48-like (OXA-48- and OXA-162-type) acquired carbapenemases DNA fragments of genes are registered separately for each type through two different channels, the results of amplification of KPC-type is registered through the fluorophore FAM, a OXA-48-like is registered through the channel of fluorophore JOE. Through the channel of fluorophore ROX the amplification product of Internal Control is detected.

Channel for fluorophore	FAM[1]	JOE[1]	ROX[1]
DNA target	KPC-type carbapenemases genes	OXA-48-like carbapenemases genes	Internal Control

3. CONTENT

AmpliSens® MDR KPC/OXA-48-FRT PCR kit is produced in 1 form:

AmpliSens® MDR KPC/OXA-48-FRT PCR kit variant FRT-100 F, **REF** R-C2(RG,CFX)-CE

AmpliSens® MDR KPC/OXA-48-FRT PCR kit variant FRT includes:

Reagent	***Discription***	***Volume, ml***	***Quantity***
PCR-mix-1-FRT KPC/OXA-48	colorless clear liquid	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 KPC/OXA-48	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 tubes

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

** add 10 µl of Internal Control during the DNA extraction procedure directly to the sample/lysis mixture (see DNA-sorb-AM, **REF** K1-12-100-CE protocol or RIBO-prep, **REF** K2-9-Et-50-CE protocol).

AmpliSens® MDR KPC/OXA-48-FRT 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
- PCR box.
- Vortex mixer.

[1] Or the similar detection channel for the detection of the indicated fluorophore in accordance with the using instrument.

- Desktop centrifuge with a rotor for 2-ml reaction tubes.
- Disposable pipette tips with filter (up to 100 µl).
- Pipettes (adjustable).
- Disposable powder-free gloves and laboratory coat.
- Tube racks (for 0.1- or 0.2-ml tube).
- Real-time instruments with five or more separated channels of fluorescence detection (for example, Rotor-Gene 6000 (Corbett Research, Australia); Rotor-Gene Q (QIAGEN, Germany); CFX 96 (Bio-Rad, USA))
- Disposable polypropylene PCR tubes (0.1- or 0.2-ml) when working with PCR kit variant FRT-100 F:
    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 aerosol 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

Obtaining samples of biological materials for PCR-analysis, transportation, and storage are described in the manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

AmpliSens® MDR KPC/OXA-48-FRT PCR kit is intended for the analysis of the genes of KPC-type, OXA-48-like (OXA-48- and OXA-162-type) acquired carbapenemases extracted with DNA extraction kits from the 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).

Sampling

6.1 *Oropharyngeal and rectal swabs*

Oropharyngeal and rectal swabs should be placed in Transport Medium for Swabs, **REF** 956-CE, **REF** 987-CE or Transport Medium with Mucolytic Agent, **REF** 952-CE, **REF** 953-CE

6.2 *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, 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 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

AmpliSens® MDR KPC/OXA-48-FRT PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. DNA extraction

It is recommended to use the following nucleic acid extraction kits:

– DNA-sorb-AM, **REF** 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, **REF** K1-12-100-CE and RIBO-prep, **REF** 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, **REF** 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**.**

Extract the DNA according to the manufacturer's protocol.

DNA extraction from each of the examined sample is carried out in the presence of I**nternal control-FL (IC)**. As a probe C- in-use reagent **Negative Control (C–)**.

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 a 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.

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 PCR

The choice of tubes for amplification depends on the type of thermo-cycler with real-time detection is being used.

To insert the reagents, DNA samples and control samples into the tubes disposable pipette tips with filters are used.

8.2.1 Preparing tubes for PCR

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

Prepare the reaction mixture just before use

The reagents are mixed on the basis of one reaction:

- **10 µl PCR-mix-1-FRT KPC/OXA-48,**
- **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.

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.

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 KPC/OXA-48,** 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 for variant FRT-100 F

	Reagent volume for specified number of reactions	
Reagent volume per one reaction, µl	**10.0**	**5.0**
Number of reactions[2]	**PCR-mix-1-FRT KPC/OXA-48**	**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**

3. In a separate tube prepare the reaction mixture. Mix appropriate amount of **PCR-mix-1-FRT KPC/OXA-48** 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.

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

6. Add **10 µl** of DNA samples obtained from the examined samples.
7. Carry out the control reactions:

C– - Add **10 µl** of **the sample extracted from the Negative Control reagent** to the tube labeled C–.

NCA - Add **10 µl** of **TE-buffer** to the tube labeled NCA (Negative control of amplification).

C+ - Add **10 µl Positive Control-2 KPC/OXA-48** to the tube labeled C+

8.3.2. Amplification

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

Table 3

AmpliSens-1 amplification program

Step	***Rotor-type instruments***[3]			***Plate-type instruments***[4]		
	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 and ROX fluorophores.

1. Insert the tubes into the reaction module of the device.
2. Run the amplification program with fluorescent signal detection.
3. Analyze results after the amplification program is completed.

9. DATA ANALYSIS

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

– The signal of the **KPC-type of carbapenemases genes** amplification product is detected through the channel for the **FAM** fluorophore.

– The signal of the **OXA-48-like of carbapenemases genes** amplification product is detected through the channel for the **JOE** fluorophore.

– The signal of the **IC** DNA amplification product is detected through the channel for the **ROX** 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 the presence (or

absence) of a *Ct* value for the DNA-target in the corresponding column of the result grid.

Principle of interpretation is the following:

- **Carbapenemases genes** are **detected** if the *Ct* value determined in the results grid in the channel for the FAM and/or JOE fluorophore is less than the boundary *Ct* value specified in the *Important Product Information Bulletin*. Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.
- **KPC-type and OXA-48-like carbapenemases genes** are **not detected** in a sample if the *Ct* value is not determined (absent) in the channels for FAM and JOE fluorophores, whereas the *Ct* value determined in the channel for the ROX fluorophore is less than the boundary *Ct* value specified in the *Important Product Information Bulletin*.
- The result is **invalid** if the *Ct* value is not determined (absent) in the channel for FAM and JOE 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.

Boundary *Ct* values are specified in the *Important Product Information Bulletin* enclosed to the PCR kit. See also Guidelines [2]

The result of the PCR is considered reliable only if the results obtained for Negative and Positive Control of amplification as well as for the Negative Control of extraction of DNA are correct according to the Table 4.

Table 4

Results for controls of different stages of the PCR

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

10. TROUBLESHOOTING

Results of the 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 and/or JOE 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 and/or JOE fluorophores. In this case the PCR should be repeated for all the samples for which the *Ct* value is defined through the channels for FAM and/or JOE, fluorophores.

The clinical interpretation of the test results should be carried out by the doctor only on the basis of complex examination of the patient according to the anamnesis data, clinical and epidemiological status, keeping into account the existed clinical and methodological recommendations.

11. TRANSPORTATION

AmpliSens® MDR KPC/OXA-48-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® MDR KPC/OXA-48-FRT** PCR kit are to be stored at 2–8 °C when not in use (except PCR-mix-2-FRT, and polymerase (TaqF)). All components of the **AmpliSens® MDR KPC/OXA-48-FRT** PCR kit are stable until the expiration date stated on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

PCR-mix-2-FRT, and polymerase (TaqF) are to be stored at the temperature from minus 24 to minus 16 °C

PCR-mix-1-FRT KPC/OXA-48 is to be kept away from light

13. SPECIFICATIONS

13.1. Sensitivity

Biological material	Transport medium	Nucleic acid extraction kit	Sensitivity, copies/ml[3]
Blood cultures, a mixture of bacterial cultures obtained by primary seeding clinical material to solid or liquid medium[4]		DNA-sorb-AM	$1x10^5$

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

[4] The bacterial cultures obtained by primary seeding clinical material onto solid medium have the sensitivity to bacterial cell suspension lysis solution "DNA-sorb-AM", respectively.

Urine	—	DNA-sorb-AM RIBO-prep	$5x10^2$
Oropharyngeal and rectal swabs	Transport Medium for Swabs" or Transport Medium with Mucolytic Agent	DNA-sorb-AM	$2x10^3$

The genes of Carbapenemases of corresponding groups were identified by using this reagents kit then the DNA samples of control strains, carrying the genes of known carbapenemases of KPC-3- and OXA-48-types, were analyzed.

13.2. Specificity

The analytical specificity of **AmpliSens® MDR KPC/OXA-48-FRT** PCR kit is ensured by 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: *Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca, Serratia marcescens, Pseudomonas aeruginosa, Acinetobacter baumannii, Proteus mirabilis, Enterococcus faecalis, Staphylococcus* spp., *Streptococcus* spp., *Candida* spp.

The clinical specificity of **AmpliSens® MDR KPC/OXA-48-FRT** PCR kit was confirmed in laboratory clinical trials.

14. REFERENCES

1. Handbook "Sampling, Transportation, and Storage of Clinical Material for PCR diagnostics", developed by Federal Budget Institute of Science "Central Research Institute for Epidemiology" of Federal Service for Surveillance on Consumers' Rights Protection and Human Well-Being, Moscow, 2010.
2. Guidelines to AmpliSens® MDR MBL-FRT and AmpliSens® MDR KPC/OXA-48-FRT PCR kit for detection of genes of *carbapenemases* by the polymerase chain reaction (PCR) with real-time hybridization-fluorescence detection developed by Federal Budget Institute of Science "Central Research Institute for Epidemiology".

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® MDR KPC/OXA-48-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

16. KEY TO SYMBOLS USED

REF	Catalogue number		Caution
LOT	Batch code		Sufficient for
RUO	Research use only		Expiration Date
VER	Version		Consult instructions for use
	Temperature limitation		Keep away from sunlight
	Manufacturer	**NCA**	Negative control of amplification
	Date of manufacture	**C–**	Negative control of extraction
C+	Positive control of amplification	**IC**	Internal control