ECOLI

eSens

CE

IVD

For Professional Use Only

eSens MRSA QT PCR kit

REF ES4300A

Instructions for Use

1 INTENDED USE

eSens MRSA QT PCR kit is an *in vitro* nucleic acid amplification test for qualitative and quantitative detection of methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* DNA and methicillin-resistant coagulase-negative *Staphylococcus* spp. DNA in the biological material (oropharyngeal swabs, bronchoalveolar lavage (BAL), sputum, endotracheal aspirate, bronchial washing fluid, urine (first portion pellet), blood, blood plasma, cerebrospinal fluid (CSF), affected organs and tissues aspirates, washes from healthcare equipment and instruments) using real-time hybridization-fluorescence detection.

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

2 PRINCIPLE OF PCR DETECTION

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

eSens MRSA QT PCR kit contains the Internal Control (Internal Control STI-87). It must be used in the extraction procedure in order to control the extraction process of each individual sample and to identify possible reaction inhibition.

eSens MRSA QT PCR kit uses “hot-start,” which greatly reduces the frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by separation of nucleotides and Taq-polymerase by a chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

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

Table 1

Channel for fluorophore	FAM	JOE	ROX
DNA-target	DNA *Staphylococcus aureus*	DNA gene *mecA*	DNA Internal Control STI-87 (IC) DNA
Target gene	Ferredoxin-dependent glutamate synthase	Target gene *mecA*	Artificially synthesized sequence

3 CONTENT

eSens MRSA QT PCR kit (ES4300A) includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-1-FRT *MRSA*	clear liquid from colorless to light lilac colour	1.2	1 tube
PCR-mix-2-FRT	colourless clear liquid	0.6	1 tube
Polymerase (TaqF)	colourless clear liquid	0.06	1 tube
DNA-buffer	colourless clear liquid	0.5	1 tube
DNA calibrator C1 *MRSA*	colourless clear liquid	0.2	1 tube
DNA calibrator C2 *MRSA*	colourless clear liquid	0.2	1 tube
Negative Control (C–)*	colourless clear liquid	1.2	2 tubes
Positive Control DNA *MRSA***	colourless clear liquid	0.1	1 tube
Internal Control STI-87 (IC)***	colourless clear liquid	0.6	2 tubes

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

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

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

eSens MRSA QT PCR kit is intended for 110 reactions (including controls).

4 ADDITIONAL REQUIREMENTS

- DNA extraction kit or the DNA extraction automatic station.
- Disposable powder-free gloves and a laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol filters (up to 100 µl).
- Tube racks.
- 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.
  c) Refrigerator at 2 to 8 °C.
- Deep-freezer at the temperature range from minus 24 to minus 16 °C.
- Reservoir for used tips.

5 GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol filters and use a new tip for every procedure.
- Store all extracted positive material (specimens, controls and amplicons) away from all other reagents and add it to the reaction mix in a distantly separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable protective gloves and laboratory cloths, and protect eyes while samples and reagents handling. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use a kit after its expiration date.
- Dispose of all specimens and unused reagents in accordance with local regulations.
- Samples should be considered potentially infectious and handled in biological cabinet in compliance with appropriate biosafety practices.
- Clean and disinfect all samples or reagents spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.
- Avoid inhalation of vapors, samples and reagents contact with the skin, eyes, and mucous membranes. Harmful if swallowed. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice if necessary.
- Safety Data Sheets (SDS) are available on request.
- Use of this product should be limited to personnel trained in DNA amplification techniques.
- Workflow in the laboratory must be one-directional, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where the previous step was performed.

⚠ Some components of this kit contain sodium azide as a preservative. Do not use metal tubing for reagent transfer.

6 SAMPLING AND HANDLING

eSens MRSA QT PCR kit is intended for the analysis of DNA extracted with DNA extraction kits from the biological material (oropharyngeal swabs, BAL, sputum, endotracheal aspirate, bronchial washing fluid, urine (first portion pellet), blood, blood plasma, CSF, puncture samples from affected organs and tissues, washes from healthcare equipment and instruments).

6.1 Blood plasma.

Tubes with collected whole blood are centrifuged at 800 g for 10 min at room temperature. Blood plasma samples of 1.0 ml volume are transferred to sterile tubes (for example, 1.5-2.0 ml Eppendorf tubes). Use tips with aerosol barriers. These tubes are centrifuged at 11,000 rpm for 10-20 min. DNA should be extracted from the pellet together with 100 µl of supernatant.

7 WORKING CONDITIONS

eSens MRSA QT 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

- **RIBO-prep** (K2-9-Et-100-CE)

- For the automatic extraction

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

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

8.2 Preparing PCR

8.2.1 Preparing tubes for PCR

The type of tubes depends on the PCR instrument used for analysis. Use disposable filter tips for adding reagents, DNA and control samples into tubes.

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

1. Prepare the mixture of **PCR-mix-2-FRT** and **polymerase (TaqF)**. To do this, transfer the entire content of the tube with **polymerase (TaqF) (60 µl)** into the tube with **PCR-mix-2-FRT (600 µl)** and carefully vortex. Avoid foaming. Centrifuge tubes for 1-2 s to remove drops from tube walls. Indicate the date of mixture preparation on the tube

NOTE: The prepared mixture is intended for 120 samples.
Store at 2–8 °C for 3 months and use as needed.

NOTE: If the mixture volume will not be utilized within 3 months it is necessary to prepare mixture for less number of reactions. For example, mix **150 µl** of **PCR-mix-2-FRT** and **15 µl** of **polymerase (TaqF)**. The obtained mixture is intended for 30 reactions.

2. Prepare the reaction mixture. Note that for analysis of even one clinical sample it is necessary to run five controls of amplification stage: two DNA calibrators (**C1 *MRSA*** and **C2 *MRSA***) in two repeats and the Negative Control of amplification (DNA-buffer). In addition, include one extra reaction when calculating reagent volumes: for detection of N samples take the reagents for N+1 reactions.

3. Mix **PCR-mix-1-FRT *MRSA*** and the mixture of **PCR-mix-2-FRT** and **polymerase (TaqF)** in a new tube in the following proportion:

   10 µl of **PCR-mix-1-FRT *MRSA*,**
   5 µl of the mixture of **PCR-mix-2-FRT** and **polymerase (TaqF)**.

One can calculate reagent volume for the needed number of reactions according to the scheme given in the Table 2.

Table 2

Scheme of reaction mixture preparation

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

21	**270**	**135**
22	**280**	**140**
23	**290**	**145**
24	**300**	**150**
25	**310**	**155**
30	**360**	**180**

***** Values are given with account of one extra reaction and five controls of amplification stage: 2 DNA calibrators, C1 *MRSA* and C2 *MRSA*, (in two replicates) and the Negative Control (DNA-buffer).

4. Take the required quantity of tubes for amplification of clinical and control DNA samples.
5. Transfer **15 µl** of the prepared mixture to each tube.
6. Add **10 µl** of **DNA** obtained from clinical or control samples to the tubes with the reaction mixture.
7. Prepare control reactions:

NCA	–	Add **10 µl** of **DNA-buffer** to the tube labeled NCA (Negative Control of amplification).
C1 MRSA **C2 MRSA**	–	Add **10 µl** of **C1 *MRSA*** to two tubes and **10 µl** of **C2 *MRSA*** to the other two tubes.
C–	–	Add **10 µl** of the sample extracted from the **Negative Control (C–)** reagent to the tube labeled C– (Negative Control of extraction).
PCE	–	Add **10 µl** of **DNA** extracted from the **Positive Control DNA *MRSA*** to the tube labeled PCE (Positive Control of extraction).

8.2.2 Amplification

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

Table 3

MRSA amplification program

	Rotor-type instruments (e.g Rotor-Gene Q or equivalent)			**Plate-type instruments** (e.g CFX 96 Touch, CFX 96 Opus, QuantStudio 5 or equivalent)		
Step	**Temperature, °C**	**Time**	**Cycles**	**Temperature, °C**	**Time**	**Cycles**
1	**95**	**15 min**	**1**	**95**	**15 min**	**1**
2	**95**	**15 s**	**5**	**95**	**15 s**	**5**
	60	**30 s**		**55**	**30 s**	
	72	**15 s**		**72**	**15 s**	
3	**95**	**15 s**	**40**	**95**	**15 s**	**40**
	55	**30 s Fluorescence acquiring**		**55**	**30 s Fluorescence acquiring**	
	72	**15 s**		**72**	**15 s**	

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

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

9 DATA ANALYSIS

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

- the amplification product of the *Staphylococcus aureus* DNA fragment is detected in the channel for the FAM fluorophore,
- the amplification product of the *mecA* gene fragment, which is located in the chromosome of *S.aureus* and some other *Staphylococcus* species in the specific region found in the methicillin-resistant strains only, is detected in the channel for the JOE fluorophore,
- the Internal Control STI-87 (IC) DNA is detected in 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 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. The principle of interpretation is the following:

Table 4

Interpretation of results and quantification for clinical samples

Channel for the fluorophore			Result (concentration calculation, copies/ml)
FAM (*S.aureus*)	**JOE (*mecA* gene)**	**ROX (IC)**	
+	**–**	**+/–**	***MSSA* (= (A/C)*IC coefficient*N)**
–	**+**	**+/–**	***MRCoNS* (= (B/C)* IC coefficient*N)**
+	**+**	**+/–**	**Calculate log A and log B.** 1. If **A** differs from **B** by not more than **0.3 log**, the result is ***MRSA* (= (B/C)* IC coefficient*N)** 2. If **A** differs from **B** by more than **0.3 log**, the result is ***MRSspp. (MRSA* and *MRCoNS)* (= (B/C)* IC coefficient*N)**
–	**–**	**–**	**Invalid**
–	**–**	**+**	**Not detected**

Where **A** – calculated concentration in the channel for the FAM fluorophore;

B – calculated concentration in the channel for the JOE fluorophore;

C – calculated concentration in the channel for the ROX fluorophore;

$$N = \frac{100}{\text{extraction volume, µl}}$$

NOTE: **IC coefficient** is specified in the *Technical Sheet* and cannot be used for calculation of results obtained with the reagents of different lot.

- **DNA of *MSSA* (methicillin-sensitive *Staphylococcus aureus*) is detected** if a *Ct* value is defined in the channel for the FAM fluorophore and there is no *Ct* value in the channel for the JOE fluorophore (the fluorescence curve does not cross the threshold line).

Concentration is calculated as follows:

(A/C)*IC coefficient*N = (copies/ml of sample)

- **DNA of *MRCoNS* (methicillin-resistant coagulase-negative *Staphylococcus* spp.) is detected** if a *Ct* value in the channel for the JOE fluorophore is defined and there is no *Ct* value in the channel for the FAM fluorophore (fluorescence curve does not cross the threshold line).

Concentration is calculated as follows:

(B/C)*IC coefficient*N = (copies/ml of a sample)

- **DNA of *MRSA* (methicillin-resistant *Staphylococcus aureus*) is detected** in a sample if the *Ct* value is defined in the channels for the FAM and JOE fluorophores. Moreover, the fluorescence curves should cross the threshold line at the area of exponential growth of fluorescence and the

difference between logarithms of calculated concentrations in the channels for the FAM and JOE fluorophores is not more than **0.3** (see Table 2). Concentration is calculated as follows:

(B/C)*IC coefficient*N = (copies/ml of sample)

If the common logarithm of calculated concentration in the channel for the FAM fluorophore differs from that in the channel for the JOE fluorophore by more than **0.3**, then the displayed result is ***"DNA of MRSspp. (MRSA and MRCoNS) (methicillin-resistant Staphylococcus spp. including methicillin-resistant Staphylococcus aureus and methicillin-resistant coagulase-negative Staphylococcus spp.)"***

Concentration is calculated as follows:

(B/C)*IC coefficient*N = (copies/ml of sample)

- Result is considered **invalid** if a *Ct* value is not defined in the results grid in the channel for the FAM fluorophore, whereas the *Ct* value in channels for the JOE and ROX channels is absent or the calculated value is less than the value specified in the *Technical Sheet*. The PCR analysis should be repeated again for such samples.

Linear measuring range of **eSens MRSA QT PCR kit** is 800–10,000,000 copies/ml. If the result is greater than 10,000,000 copies/ml, it is indicated as ***the result is greater than 10,000,000 copies/ml***. If the result is less than 800 copies/ml, it is indicated as ***the result is less than 800 copies/ml.***

NOTE: Concentration values of DNA calibrators are specified in the *Technical Sheet*.

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

Table 5

Results for controls

Control	Stage for control	Result of amplification in the channel for the fluorophore		
		FAM	JOE	ROX
C–	DNA extraction	Absent	Absent	> boundary value
PCE	DNA extraction, PCR	Value is within the range	Value is within the range	> boundary value
NCA	PCR	Absent	Absent	Absent
K1 *MRSA* K2 *MRSA*	PCR	*Ct* value and calculated concentration are determined	*Ct* value and calculated concentration are determined	*Ct* value and calculated concentration are determined

NOTE: Boundary *Ct* values and concentration range of PCE are specified in the *Technical Sheet* enclosed to the PCR kit.

10 TROUBLESHOOTING

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

1. If an **invalid** result is obtained it is necessary to repeat PCR analysis of the required biological sample.
2. Absence of positive signal in DNA calibrators can result from incorrect settings of amplification program or failures of PCR preparation. In that case it is necessary to repeat PCR again for all samples.
3. If a positive signal is detected for the Negative Control of extraction (C–) in the FAM and/or JOE channels and for the Negative Control of amplification (NCA) in any of the channels, FAM, JOE and/or ROX, it means that contamination of reagents or samples has occurred. In that case results for all samples are considered to be invalid. The analysis must be repeated and measures for detecting and eliminating the contamination source must be taken.
4. If a positive result is detected for a test sample, whereas its fluorescent curve does not have exponential slope (it more looks like straight line), it means that the threshold or baseline parameters are set incorrectly. This result cannot be considered as positive. If the threshold value was correct it is necessary to repeat PCR for this sample.

11 TRANSPORTATION

eSens MRSA QT PCR kit should be transported at 2–8 °C for no longer than 5 days.

12 STABILITY AND STORAGE

All components of the **eSens MRSA QT PCR kit** are to be stored at 2–8 °C when not in use (except for PCR-mix-1-FRT *MRSA*, PCR-mix-2-FRT, and polymerase (TaqF)). All components of the **eSens MRSA QT 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-1-FRT *MRSA*, PCR-mix-2-FRT, and polymerase (TaqF) are to be stored at temperature from minus 24 to minus 16 °C.

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

13 SPECIFICATIONS

13.1 Sensitivity

Table 6

Test material	Nucleic acid extraction kit	Analytical sensitivity, copies/ml	Linear measuring range, copies/ml
oropharyngeal swabs, BAL, sputum, endotracheal aspirate, bronchial washing fluid, urine*, blood, blood plasma, CSF, puncture samples from affected organs and tissues, washes from healthcare equipment and instruments	RIBO-prep	400	800 – 10,000,000

* Pretreatment is required.

13.2 Analytical specificity

The analytical specificity of **eSens MRSA QT 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 specific activity of **eSens MRSA QT PCR kit** was confirmed in studies of bacterial strains of *Staphylococcus aureus* including *MRSA*, as well as by analyzing clinical material with subsequent confirmation of results by sequencing the amplified fragments.

Analytical specificity was tested on the following strains and isolates: *Chlamydophila pneumonia*, *Escherichia coli*, *Haemophilus haemolyticus*, *H.influenzae*, *H.parainfluenzae*, *Klebsiella oxytoca*, *K.pneumonia*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium tuberculosis*, *Mycoplasma pneumonia*, *Neisseria cinereae*, *N.elongata*, *N.flavescens*, *N.gonorrhoeae*, *N. meningitidis*, *N.mucosa*, *N.sicca*, *N.subflava*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Shigella flexneri*, *Streptococcus agalactiae*, *S.milleri*, *S.mitis*, *S.mutans*, *S. pneumoniae*, *S.pyogenes*, *S.salivarius*, *S.sanguis*, *S.suis*, *S.viridans*, as well as human genome DNA. Testing the above-mentioned strains with this PCR kit did not reveal nonspecific responses.

The clinical specificity of **eSens MRSA QT PCR kit** was confirmed in laboratory clinical trials.

14 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 **eSens MRSA QT PCR kit** has been tested against predetermined specifications to ensure consistent product quality.

15 KEY TO SYMBOLS USED

REF	Catalogue number	⚠	Caution
LOT	Batch code	Σ	Contains sufficient for <n> tests
IVD	In vitro diagnostic medical device		Use-by Date
VER	Version		Consult instructions for use
	Temperature limit		Keep away from sunlight
	Manufacturer	**NCA**	Negative control of amplification
	Date of manufacture	**C-**	Negative control of extraction
EC REP	Authorized representative in the European Community	**PCE**	Positive control of amplification
MRSA	Methicillin-resistant Staphylococcus aureus	**C1 *MRSA*, C2 *MRSA***	DNA Calibrators
MSSA	Methicillin-sensitive Staphylococcus aureus		
MRCoNS	Methicillin-resistant coagulase-negative Staphylococcus spp.		

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
01_04/2022		

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