

AmpliSens® MRSA-screen-titre-FRT PCR kit



For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

	Catalogue number		Caution
	Batch code		Contains sufficient for <n> tests
	In vitro diagnostic medical device		Use-by Date
	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
	Authorized representative in the European Community	PCE	Positive control of amplification
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>	C1 MRSA, C2 MRSA	DNA Calibrators
MSSA	Methicillin-sensitive <i>Staphylococcus aureus</i>	MRSspp.	Methicillin-resistant <i>Staphylococcus</i> spp.
MRCoNS	Methicillin-resistant coagulase-negative <i>Staphylococcus</i> spp.		

1. INTENDED USE

AmpliSens® MRSA-screen-titre-FRT 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.

AmpliSens® MRSA-screen-titre-FRT 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.

AmpliSens® MRSA-screen-titre-FRT 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 <i>Staphylococcus aureus</i>	DNA gene <i>mecA</i>	DNA Internal Control STI-87 (IC) DNA
Target gene	Ferredoxin-dependent glutamate synthase	Target gene <i>mecA</i>	Artificially synthesized sequence

3. CONTENT

AmpliSens® MRSA-screen-titre-FRT PCR kit is produced in 1 form: variant FRT-100 F, R-B78-100-FT(RG,iQ)-CE

Variant FRT-100 F 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 (see RIBO-prep K2-9-Et-100-CE).

Variant FRT-100 F 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 3000/6000 (Corbett Research, Australia); Rotor-Gene Q (QIAGEN, Germany); iCycler iQ5 (Bio-Rad, USA)).
- Disposable polypropylene PCR tubes (0.2- or 0.1-ml):
 - 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 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

NOTE: 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® MRSA-screen-titre-FRT 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).

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

AmpliSens® MRSA-screen-titre-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:

— **RIBO-prep**, **REF** K2-9-Et-100-CE,

— NucliSENS easyMAG automated system (for details see Guidelines [2]).

The RNA extraction of each test sample is carried out in the presence of **Internal Control STI-87-rec (IC)**.

In the extraction procedure it is necessary to carry out the control reaction as follows:

C– — Add **100 µl of Negative Control (C–)** to the tube labelled C– (Negative Control of Extraction).

PCE — Add **90 µl of Negative Control (C–)** and **10 µl of Positive Control DNA MRSA** to the tube labelled PCE (Positive Control of Extraction).

NOTE: Extract DNA according to the manufacturer's protocol.

8.2. Preparing PCR

8.2.1. Preparing tubes for PCR

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

The type of tubes depends on the type of PCR real-time instrument.

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

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.

If the mixture volume will not be utilized within 3 months it is necessary to

NOTE: 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 1 reaction, µl	Reagent volume for specified number of reactions, µl	
	10.0	5.0
Number of biological samples	PCR-mix-1-FRT MRSA ¹	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

¹ 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).

- Take the required quantity of tubes for amplification of clinical and control DNA samples.
 - Transfer **15 µl** of the prepared mixture to each tube.
 - Add **10 µl of DNA** obtained from clinical or control samples to the tubes with the reaction mixture.
 - Prepare control reactions:**
- NCA** — Add **10 µl of DNA-buffer** to the tube labeled NCA (Negative Control of amplification).
- C1 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

Step	Rotor-type instruments ²			Plate-type instruments ³		
	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		55	30 s	
		Fluorescence acquiring			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;

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

NOTE: **IC coefficient** is specified in the *Important Product Information Bulletin* 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:

$$(A/C)*IC \text{ coefficient} * N = (\text{copies/ml of sample})$$

– 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:

$$(B/C)*IC \text{ coefficient} * N = (\text{copies/ml of a sample})$$

– 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:

$$(B/C)*IC \text{ coefficient} * N = (\text{copies/ml of sample})$$

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

² For example, Rotor-Gene 3000, Rotor-Gene 6000 (Corbett Research, Australia), Rotor-Gene QiAGEN, Germany).

³ For example, iCycler iQ, iCycler iQ5 (Bio-Rad, USA), Mx3000P (Stratagene, USA).

result is "DNA of MRSpp. (MRSA and MRCoNS) (methicillin-resistant *Staphylococcus* spp. including methicillin-resistant *Staphylococcus aureus* and methicillin-resistant coagulase-negative *Staphylococcus* spp.)"

Concentration is calculated as follows:

$$(B/C)^{1/C} \text{ coefficient} * N = (\text{copies/ml of sample})$$

- 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 *Important Product Information Bulletin*. The PCR analysis should be repeated again for such samples.

Linear measuring range of AmpliSens® MRSA-screen-titre-FRT 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 *Important Product Information Bulletin*.

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).

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 *Important Product Information Bulletin* enclosed to the PCR kit. See also Guidelines [2].

10. TROUBLESHOOTING

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

- If an **invalid** result is obtained it is necessary to repeat PCR analysis of the required biological sample.
- 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.
- 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.
- 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.

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

11. TRANSPORTATION

AmpliSens® MRSA-screen-titre-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® MRSA-screen-titre-FRT 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 AmpliSens® MRSA-screen-titre-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-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
<ul style="list-style-type: none"> oropharyngeal swabs, BAL, sputum, endotracheal aspirate, bronchial washing fluid, urine⁴, blood, blood plasma, CSF, puncture samples from affected organs and tissues, washes from healthcare equipment and instruments 	RIBO-prep	400	800 – 10,000,000

⁴ Pretreatment is required.

13.2. Analytical specificity

The analytical specificity of AmpliSens® MRSA-screen-titre-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 specific activity of AmpliSens® MRSA-screen-titre-FRT 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: *Chlamydomyphila pneumoniae*, *Escherichia coli*, *Haemophilus haemolyticus*, *H.influenzae*, *H.parainfluenzae*, *Klebsiella oxytoca*, *K.pneumoniae*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycobacterium tuberculosis*, *Mycoplasma pneumoniae*, *Neisseria cinerea*, *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 AmpliSens® MRSA-screen-titre-FRT PCR kit was confirmed in laboratory clinical trials.

14. REFERENCES

- 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.
- Guidelines to the AmpliSens® MRSA-screen-titre-FRT PCR kit 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 by 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® MRSA-screen-titre-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
29.04.15 ME	Through the text	Corrections according to the template
12.02.18 PM	3. Content	The colour of the reagent was specified
14.07.20 KK	Through the text	The text formatting was changed
	2. Principle of PCR detection Footer	The table with targets was added 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

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