Package insert

GeneProof
Molecular diagnostics for your routine

GeneProof MRSA PCR Kit

CE IVD

In vitro diagnostic medical device

The kit has been manufactured according to EC Directive 98/79/EC as an *in vitro* diagnostic medical device and it has been designed for professional use in specialized clinical and research laboratories.

KIT CONTENT

	ISIN Version IS included in the MasterMix			ISEX Version IS supplied in a separate tube Nucleic acid extraction and PCR inhibition control		
REF	**MRSA/ISIN/025** 25 rxn	**MRSA/ISIN/050** 50 rxn	**MRSA/ISIN/100** 100 rxn	**MRSA/ISEX/025** 25 rxn	**MRSA/ISEX/050** 50 rxn	**MRSA/ISEX/100** 100 rxn
MasterMix MRSA	1x750 µl	2x750 µl	4x750 µl	1x750 µl	2x750 µl	4x750 µl
Positive Control MRSA	1x200 µl	1x200 µl	2x200 µl	1x200 µl	1x200 µl	2x200 µl
Internal Standard MRSA	-	-	-	1x1000 µl	1x1000 µl	2x1000 µl

STORAGE AND TRANSPORTATION CONDITIONS

The kits could be transported at temperature below -20 °C. The kit will remain stable at least until the expiry date printed on the package, if the storage temperature is kept (-20 ± 5 °C). Kit is stable after 15 repeated freezing/thawing cycles.

TECHNICAL SPECIFICATION

Target Sequence	*nuc* gene, which is specific for *Staphylococcus aureus*, SCCmec/orfX junction region, resistance gene *mecA/mecC*
Specificity	Methicillin-resistant *Staphylococcus aureus* (MRSA)
Sensitivity (LoD)	Reaches up to 2.81 ge/µl with the probability of 95% (on NCTC strain 13142)
Validated specimens	aspirate, sputum, swab, urine
External Quality Assessment	Regularly tested by the QCMD and Instand e.V. External Quality Assessment Panels
Regulatory status	CE IVD

Quality management system is certified in compliance with the requirements of the standard ISO 13485:2016

METHOD PRINCIPLES

The PCR kit is designed for methicillin-resistant *Staphylococcus aureus* detection by the real-time Polymerase Chain Reaction (PCR) method. The MRSA detection consists in amplification of three specific loci (the *nuc* gene which is specific for *Staphylococcus aureus*, the SCCmec/orfX junction region and the resistance genes *mecA/mecC*) and in measurement of fluorescence increase. The *nuc* gene presence is indicated by the TexRed fluorophore fluorescence growth. The SCCmec/orfX junction presence is indicated by the FAM fluorophore fluorescence growth. The resistance gene *mecA/mecC* presence is indicated by the Cy5 fluorophore fluorescence growth. An Internal Standard (IS) is either included in the reaction mix, controlling the possible inhibition of the PCR (ISIN version) or excluded, controlling also the DNA extraction process quality (ISEX version). IS positive amplification is detected in the HEX fluorophore fluorescence channel. The detection kit takes advantage of the "hot start" technology, minimizing non-specific reactions and assuring maximum sensitivity. Ready to Use MasterMix contains uracil-DNA-glycosylase (UDG), eliminating possible contamination of the PCR by amplification products. The kit is designed for *in vitro* diagnostics and provides qualitative detection.

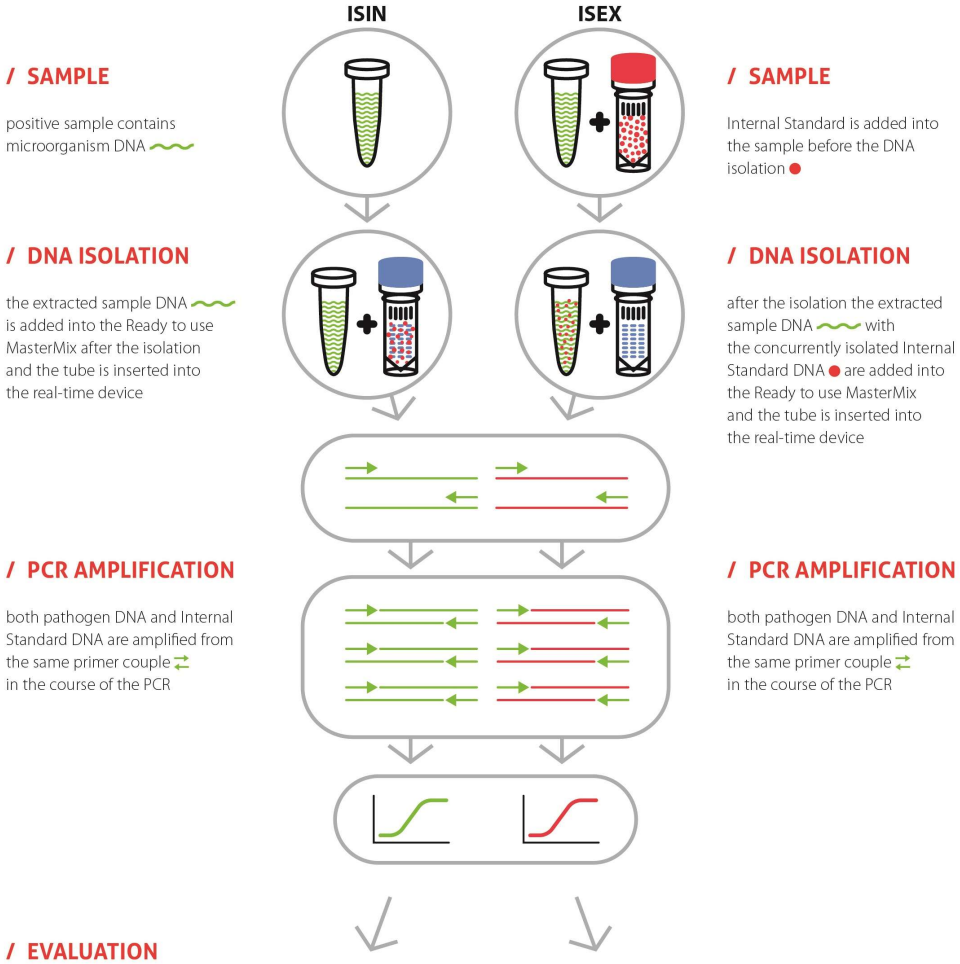
ISIN version

Internal Standard is included in the MasterMix tube. This PCR kit version enables PCR inhibition control.

ISEX version

Internal Standard is provided as independent item within the package. This PCR kit version enables both PCR inhibition control and nucleic acid extraction process efficiency control.

MICROBIOLOGICAL DNA DETECTION TECHNOLOGY



USER MANUAL

SAMPLING AND SAMPLE STORAGE

Sampling of all sample types (aspirate, sputum, swab and urine) should be performed into sterile tubes without any transportation media and the samples should be transported within 24 hours at the temperature between +2 °C and +8 °C. In case of longer storage keep all samples frozen at the temperature below -10 °C.

NUCLEIC ACID PURIFICATION

Nucleic acid extraction should be performed by extraction kits available at the market according to protocols for the particular clinical material extraction. The manufacturer recommends the following extraction kits:

croBEE NA16 Nucleic Acid Extraction System
GeneProof PathogenFree DNA Isolation Kit

When using the ISEX versions of the PCR kits the IS should be added directly into the sample at the beginning of the isolation process so that in the end 1 µl of the resulting elution volume contains 0.1 µl of the IS:

Elution volume	**25 µl**	**50 µl**	**100 µl**	**200 µl**
Internal Standard	2,5 µl	5 µl	10 µl	20 µl

PCR SETUP

1. Add 30 µl of MasterMix into PCR tubes.

2. Add 10 µl of the isolated nucleic acid sample or 10 µl of Calibrator/Positive Control into the individual PCR tubes. The final reaction mix volume will be 40 µl. *It is necessary to keep all components at +2 °C to +8 °C during the PCR preparation.*

3. Close the tubes, centrifuge shortly, insert them into the device and let them amplify according to the following PCR profile.
Be very careful when handling the Calibrator/Positive Control or the clinical material, incorrect handling could result in contamination and the consequent impairment of the kit components or the MasterMix! The manufacturer is not responsible for the kit impairment due to incorrect handling.

AMPLIFICATION PROFILE

Step	Temperature	Time	Data Collection	Cycles
Hold	37 °C	2 min		1
Hold	95 °C	10 min		1
PCR	95 °C	5 s	FAM+HEX+Cy5+TexRed	45
	60 °C	40 s		
	72 °C	20 s		

INSTRUMENTS

GeneProof MRSA PCR Kit is designed for use with real-time devices from various manufacturers:

	SCCmec/orfX	**IC**	***mecA/mecC***	***nuc***
croBEE Real-Time PCR System	FAM	HEX	Cy5	TexRed
Applied Biosystems 7500 Real-Time PCR System	FAM	JOE	Cy5	TexRed
AriaMx Real-Time PCR System	FAM	HEX	Cy5	TexRed
CFX96™/Dx Real-Time PCR Detection System	FAM	HEX	Cy5	TexRed
LineGene 9600/9600 Plus	FAM	HEX	Cy5	TexRed
QuantStudio™ 5 Real-Time PCR System	FAM	VIC	Cy5	ROX
Rotor-Gene 3000 / Q	FAM	JOE	Cy5	ROX

Required detection channels: FAM, HEX, Cy5, TexRed

GeneProof diagnostic kits are continually verified with various types of devices. Current list is available at www.geneproof.com or request the list at support@geneproof.com.

CLINICAL SAMPLE ANALYSIS EVALUATION

Channel 1	Channel 2	Channel 3	Channel 4	Interpretation
FAM	TexRed/ ROX	Cy5	HEX/JOE/VIC	
SCCmec/orfX junction	*nuc*	*mecA/mecC*	IC	
+	+	+	+/-	*MRSA positive**
-	+	+	+/-	*MRSA negative***
+	-	-	+/-	*MRSA negative*
-	+	-	+/-	*MRSA negative*
-	-	+	+/-	*MRSA negative*
+	+	-	+/-	*MRSA negative*
+	-	+	+/-	*MRSA negative*
-	-	-	+	*MRSA negative*
-	-	-	-	**invalid**

* All channels 1-3 must be positive.

**In case of new MRSA variant presence, channel FAM could be negative

note 1: Sample that contains a mixture of multiple pathogens can give a false positive result.

WARNING

A single valid package insert for a specific kit is included in the package or to be requested for the particular lot from the manufacturer. The kit should be disposed of after use according to the current legal regulations considering the fact that the kit does not contain any dangerous, infectious or toxic components that would be subject to special safety regulations, and the packaging materials are made of paper and polypropylene. If you have any questions please contact our Customer Service.

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