



**Rons' MRSA Screen
Methicillin-resistant *Staphylococcus aureus*
Diagnostic Kit for real-time PCR**

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1. Reagents and Materials

1.1 Test Kit Components

Instruction manual

Primer/Probe/Nucleotide Mix

primer set, probe and deoxynucleotide triphosphates dATP, dCTP, dGTP and dUTP, aliquoted for 25 reactions, lyophilized

red cap (Reac B)

Internal Control Probe

probe, aliquoted for 25 reactions, lyophilized

yellow cap (Reac C)

PCR 10x Reaction Buffer

500 µl

blue cap (Reac A)

positive control

DNA-fragment of methicillin-resistant *Staphylococcus aureus* prepared by PCR, non-infectious, lyophilized

green cap (positive control)

Internal control

Plasmid DNA, non-infectious, lyophilized

yellow cap (internal control)

1.2 Stability and Storage

Kit components are stable during shipping. Upon receipt, store at +2°C to +8°C. After rehydration of the *Primer/Probe/Nucleotide Mix*, the *Internal Control Probe*, the *Positive Control* and the *Internal Control*, store below -18°C and avoid repeated freezing and thawing. Protect the *Primer/Probe/Nucleotide Mix* and *Internal Control Probe* from light. For repeated testing of low sample numbers, *Primer/Probe/Nucleotide Mix*, *Internal Control Probe* and controls should be aliquoted after rehydration. By following these recommendations, the kit is stable until the expiration date stated on the boxlabel or the Guarantee Certificate.

1.3 Supplemental Requirements

real-time machine suitable with ROX and FAM dyes

glass capillaries or PCR reaction tubes

microcentrifuge, micropipettes and filtered tips

deionized, DNA-free water

Polymerase

The test provides excellent results with Bioron's S-Taq DNA-Polymerase.

Note: We can neither guarantee a high level of sensitivity nor compatibility with other polymerases. If you intend to test our S-Taq DNA-Polymerase in parallel with your in-house polymerase, please feel free to contact us and get a gratis S-Taq sample. However, if you want to use your own polymerase, it may be necessary to use the specific buffer provided with this polymerase.

Catalog #	Pack size
CE93002	2x25 units (for more than 25 rcs)
CE93004	3x25 units (for more than 75 rcs)
CE93008	6x25 Units (for more than 150 rcs)

Do not substitute the kit buffer when using a real-time instrument with glass capillary system.



2. Application and Test Principle

Rons' MRSA Screen is an *in vitro* test for quantitative and rapid real time PCR-based diagnostics of Methicillin-resistant *Staphylococcus aureus* in clinical samples. Detection requires less than 50 MRSA per sample volume. The primer set and probe are specific for a segment the *mecA* gene coding for the Methicillin resistance. The *mecA* gene is also present in other *Staphylococcus* species like *S. epidermidis*, *S. intermedius* and *S. pseudointermedius*. *mecA* within these species will also be detected. The target probe emits fluorescent light at 520 nm.

Rons' MRSA Screen also provides internal control DNA, which can be added to the reaction. When running the PCR with the internal control DNA, a successfully performed reaction with a negative result is indicated by a distinct fluorescent signal. The internal control is detected by another probe emitting light at 600 nm.

The heat-labile Uracil-DNA Glycosylase (UDG) is suitable for preventing carry-over contamination between PCRs. This technique relies on the incorporation of deoxyuridine triphosphate (dUTP) during all amplification reactions and the pretreatment of all successive PCR mixtures with the heat-labile UDG. The UNG cleaves DNA at any site where a deoxyuridylate residue has been incorporated. Subsequently, the resulting abasic sites are hydrolyzed due to the high temperature during the initial denaturation step, and cannot serve as PCR templates any longer. The heat-labile UNG is inactivated at the same time. Native DNA (e.g., the template DNA) does not contain Uracil and is therefore not degraded by this procedure. UDG is not provided with Rons' MRSA Screen but can be ordered at Bioron with product-no: 111005 (200 units).

3. Test Protocol

3.1 Preparation of Sample Material

Clinical samples should be from all kinds of infection from the patients. Primarily swabs from skin infections, furuncles, ichors and blood as well as medical equipment such as catheter. The swab probes can be analyzed immediately or after the preceding cultivation. The swabs should be sterile and without medium (e.g. Darcan®). It is necessary templates for PCR analysis prepared by DNA extraction using a DNA extraction kit. 2 µl of the DNA extract can be used directly as PCR template. The extracts can be stored at a temperature of below -18°C for a period of one year. Repeated freezing and thawing, or storage in the refrigerator for more than 12 hours should be avoided. The sample should not contain more than 100 µg/ml DNA. To avoid false positive results, we recommend the use of deionized, DNA-free Water.

3.2 Rehydration of the Reagents

1. centrifuge tubes with lyophilized components (5 sec at maximum speed)
2. add appropriate amount of deionized, DNA-free water

Primer/Probe/Nucleotide Mix	65 µl
Internal Control Probe	65 µl
Positive Control DNA	300 µl
Internal Control DNA	300 µl

incubate for 5 minutes at room temperature
vortex and centrifuge again

Keep reagents on ice and store below -18°C after rehydration.



3.3 Experimental Protocols for real time PCR Instruments

e.g. *LightCycler*[®] I / II

Program 1: Pre-incubation

Cycles	1
Analysis Mode	None
Temperature Targets [°C]	Segment 1
Target Temperature [°C]	95
Incubation time [min]	2:00
Temperature Transition Rate [°C/s]	20.0
Secondary Target Temperature [°C]	0
Step Size [°C]	0.0
Step Delay [Cycles]	0
Acquisition Mode	None

The incubation time depends on the polymerase used. Hot start enzymes need to be activated at 95°C. Please see polymerase data sheet for duration.

Program 2: Amplification

Cycles	45			
Analysis Mode	Quantification			
Temperature Targets [°C]	Segment 1	Segment 2	Segment 3	Segment 4
Target Temperature [°C]	95	55	60	72
Incubation time [s]	0	5	7	5
Temperature Transition Rate [°C/s]	20.0	20.0	20.0	20.0
Secondary Target Temperature [°C]	0	0	0	0
Step Size [°C]	0.0	0.0	0.0	0.0
Step Delay [Cycles]	0	0	0	0
Acquisition Mode	None	None	Single	None

Segment 3 is essential for the performance of the kit. Do not skip or modify this step.

Program 3: Cooling

Cycles	1
Analysis Mode	None
Temperature Targets [°C]	Segment 1
Target Temperature [°C]	40
Incubation time [s]	30
Temperature Transition Rate [°C/s]	20.0
Secondary Target Temperature [°C]	0
Step Size [°C]	0.0
Step Delay [Cycles]	0
Acquisition Mode	None



3.4 The PCR Master Mix

Total volume per reaction is 25 µl. When setting up reactions, calculations should also include positive and negative controls. Pipet master mix on ice into a 1.5 ml reaction tube and mix gently.

Pipetting schemes:

	for 1 reaction	for 25 reactions*
water	9.8 µl	245.0 µl
10x reaction buffer (blue cap)	2.5 µl	62.5 µl
primer/probe/nucleotide mix (red cap)	2.5 µl	62.5 µl
internal control probe (yellow cap)	2.5 µl	62.5 µl
internal control (yellow cap)	2.5 µl	62.5 µl
polymerase (5 U/µl)	0.2 µl	5.0 µl

* the equivalent of the content of one red-capped vial

For other enzyme concentrations the amount of enzyme and the amount of water added to the mix need to be adjusted. Aliquot 23 µl of master mix into each PCR reaction tube.

The total duration from master mix preparation to PCR cycling must not exceed 45 minutes to avoid a decrease in the fluorescent signal.

Add 2 µl of sample (as described above) to PCR reaction tube per sample being tested. After pipetting the negative control (2 µl of water /reaction), the tube must be sealed before proceeding with the samples. Also pipetting of the samples and sealing the tubes must be completed before proceeding with the positive control (2 µl/reaction) in order to avoid cross contamination.

3.5 Test Evaluation

The analysis of the obtained data is divided into two parts:

quantitative analysis of methicillin resistant *Staphylococcus aureus* DNA in fluorescence channel F1 or FAM-specific signal with a Ct value of > 36.

qualitative analysis of Internal Control DNA in fluorescence channel F2 or ROX-specific signal

3.5.1 Data analysis with *Lightcycler II*

The internal control signals are weakly visible in channel 610 nm. Please use the following procedure to a better presentation:

„Select channel 610 nm“

„Select channel denominator 530“

single click the Y-axis with the right mouse button and choose chart preference to change the scale of the axis

deselect „Y-automatic“ and fill in 0-2

at last please save your changes with „safe“

The recalculated data have to be interpreted in the following way:

negative control and negative samples: increase of fluorescence between crossing threshold 31-35

positive control and positive sample: decrease of fluorescence (only in this mode)

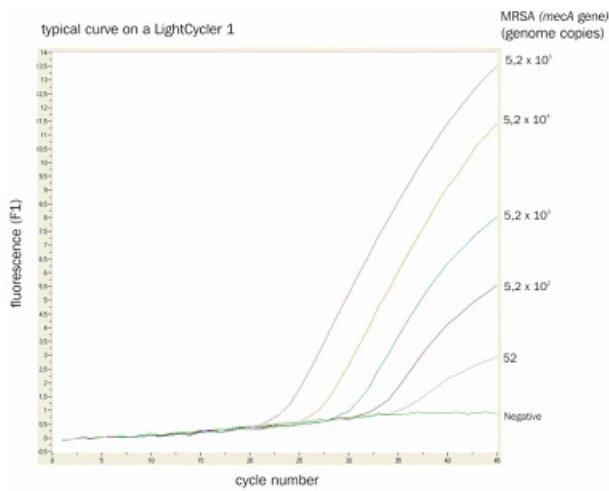
inhibited samples: no in- or decrease of fluorescence, PCR is inhibited



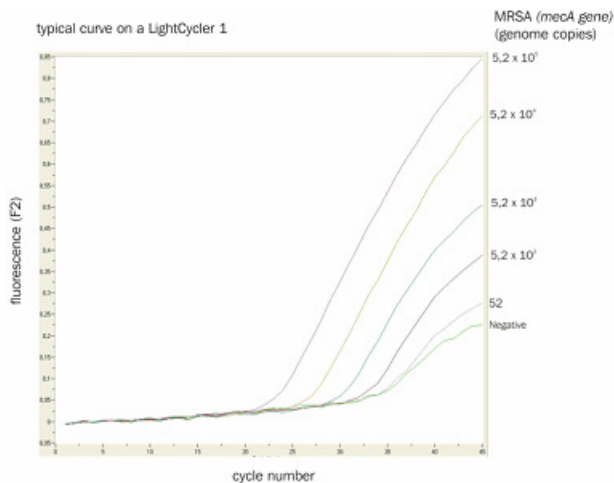
The ENZYME Company

A MRSA contamination of the sample is indicated by an increasing fluorescence signal in channel F1 during PCR. The concentration of the contaminant can be calculated by a software comparing the crossing cycle number of the sample with a standard curve created in the same run.

A successfully performed PCR without inhibition is indicated by an increasing fluorescence signal in channel F2, provided the Internal Control is added to the master mix. MRSA DNA and Internal Control DNA are competitors in PCR. Because of the very low concentration of Internal Control in the PCR mix, the signal in channel F2 is reduced with MRSA DNA loads in the sample of >10 copies/test.



Amplified dilution series of approx. $5,2 \times 10^5$, $5,2 \times 10^4$, $5,2 \times 10^3$, $5,2 \times 10^2$ and 52 genome equivalents of MRSA (*mecA* gene) as starting template. As a negative control, the template DNA was replaced with PCR-grade water. The fluorescence channel was set to F1.



In the same run the amplification of Internal Control DNA was shown in channel F2.

Amplified Internal Control DNA and a dilution series of MRSA (*mecA* gene) as starting template. As a negative control, the MRSA DNA was replaced with PCR-grade water. The fluorescence channel was set to F2.

3.5.2 Trouble Shooting

No amplification of control DNA may be due to the following reasons:

- activity of *Taq* polymerase is insufficient

- control DNA tubes have not been spun down before rehydration

- programming mistake

- pipetting mistake

Before run of a negative and a positive control please check Cycler protocol and pipetting scheme. When using polymerases other than the S-TAQ DNA Polymerase, please note the comments made under chapter 1.3. The concentration can then be raised up to 2.5 U/reaction. Please note the complete change of the pipetting scheme.



Appendix

Limited Product Warranty

This warranty limits our liability for replacement of this product. No warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided. Bioron shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

Notice to Purchaser

This product is optimized for use in the Polymerase Chain Reaction („PCR“) covered by patents owned by Hoffmann-La Roche, Inc., and F. Hoffmann-La Roche Ltd. („Roche“). No license under these patents to use the PCR process is conveyed expressly or by implication to the purchaser of this product. Bioron does not encourage or support the unauthorized or unlicensed use of the PCR process. Use of this product is restricted to persons that either have a license to perform PCR or are not required to obtain a license.

Limited License

The use of this product for the detection of Methicillin-resistant *Staphylococcus aureus* infections is covered by a Scorpions sub-license from DxS Ltd. Further information on Scorpions licenses can be obtained from DxS Ltd, 48 Grafton St, Manchester, UK M13 9XX.

EG-Konformitätserklärung/EC Conformity Declaration

Bioron GmbH, Rheingönheimerstrasse 36, 67065 Ludwigshafen

Der bezeichnete Kit entspricht den einschlägigen grundlegenden Anforderungen der aufgeführten EG-Richtlinien und Normen.
Bei einer nicht mit uns abgestimmten Änderung des Kits verliert diese Erklärung ihre Gültigkeit.

The device named below fulfills the relevant fundamental requirements of
the EC directives and standards listed. In the case of unauthorized modifications to the device, this declaration becomes
invalid.

Produktbezeichnung, Device name: **Rons' MRSA Testkit**

Produkttyp, Device type: Methicillin-resistent *Staphylococcus aureus* **Diagnostic Kit for real-time PCR**

Einschlägige EU-Richtlinien, Relevant EC directives: **EU-Richtlinie 98/79/EG für In-Vitro-Diagnostika vom 27.10.1998**

20.08.2006

Ludwigshafen, Datum

Geschäftsführung, Managing Director

Projektmanagement, Project Management