

Quick Start Protocol

FBC111 MRSA direct PCR



Test for the direct qualitative detection of methicillin-resistant *Staphylococcus aureus* (MRSA) on a qPCR cycler

Read the entire Instructions for Use (IFU) and follow them carefully before performing the test.

Deviations from the given test protocol can lead to incorrect results. Good laboratory practice should be followed during the test.

Intended Use

The MRSA direct PCR test is an *in vitro* real-time polymerase chain reaction (qPCR) assay for the qualitative detection of methicillin-resistant *Staphylococcus aureus* from nasal swabs. The test is intended to support the diagnosis as well as the prevention and control of MRSA infections in healthcare settings. Negative results do not rule out MRSA infection and should not be used as the sole basis for diagnosis. The test is intended for use in qualified laboratories by personnel trained in molecular diagnostic techniques.

Package Content

Vials with solution A (blue lid; 1.5 mL) and solution B (yellow lid; 100 µL), each for one microtiter plate (96 well; not included), Positive Control PC (green lid; 13 µL; optional), Negative Control NC (colourless lid; 13 µL; optional), Instructions for Use.

Notes before starting

The starting material for the MRSA direct qPCR test is **10 µL/reaction solubilised patient sample** directly used for qPCR without any preparatory steps (DNA isolation). Optionally, the solubilized sample can be mixed 1:1 with molecular grade water and/or be heated at 95 °C for 5 minutes before it is added to the reaction solution. Patient samples in solutions with chaotropic salts such as guanidinium thiocyanate must not be used. One positive and one negative control should be included in each PCR run.

Material provided by user

- Adequate pipettes and sterile filter-tips for PCR testing (DNase/RNase-free)
- qPCR microtiter plate or reaction tubes plus lids/adhesive optical film
- qPCR instrument (e.g. LightCycler 480; Biorad CFX)
- Disposable protective gloves, powder-free
- Table centrifuge

Test procedure

1. Thaw all reagents completely and keep them cool (+2 °C to +8 °C) directly before starting the test, use within 4 hours.
2. Optional: mix sample 1:1 with molecular grade water
3. Optional: heat sample at 95 °C for 5 minutes
4. Prepare the reaction solution: add 100 µL of solution B to the vial of solution A; mix/shake briefly and centrifuge if necessary. Do not vortex!
5. Pipette 15 µL/well of the reaction solution into each PCR reaction tube/well of the microtiter plate.
6. Add 10 µL/well of a solubilised patient sample and 10 µL Positive Control as well as Negative Control in the respective wells.
7. Close the microtiter plate with an adhesive optical film or the reaction tubes with the lids provided .
8. Briefly centrifuge the microtiter plates or reaction tubes.
9. Place the filled plate/reaction tubes in the qPCR cycler. Start program.



Instrument settings

| Steps | Temperature [°C] | Time | Number of cycles |
|--------------------------|------------------|--------|------------------|
| Cell lysis | 37 | 2 min | 1x |
| Initial denaturation | 95 | 5 min | 1x |
| Denaturation | 95 | 10 sec | 45x |
| Amplification/Elongation | 60 | 30 sec | |

Channel settings for FBC111

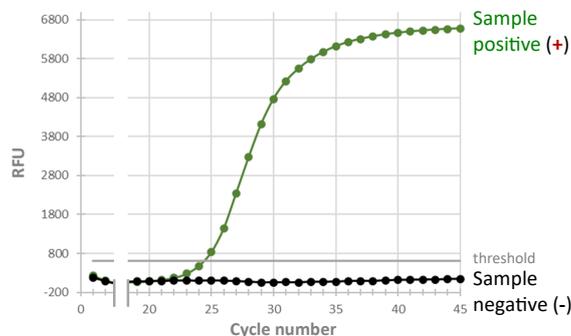
| | MREJ | IC | mecC | mecA |
|---------------|---------------------|--------------|---------|------|
| Reporter dye | FAM | HEX | Red 610 | Cy5 |
| Colour | green | yellow-green | orange | red |
| Emission [nm] | 520 | 560 | 610 | 670 |
| Quencher | Black Hole Quencher | | | |

Interpretation of test results

Positive samples (+) show a qPCR typical amplification curve that crosses a certain threshold generating the Ct value.

The results are used to identify methicillin-resistant *Staphylococcus aureus* DNA. Positive results are an indication of the presence of the pathogen. A negative result does not rule out the presence of the bacterium, as the results depend on correct sampling and a sufficient amount of DNA to be detected.

| MREJ | mecC | mecA | IC | Result | Interpretation |
|------|------|------|-----|---------|---------------------------------------|
| + | + | - | +/- | Valid | MRSA detected, positive for mecC. |
| + | - | + | +/- | Valid | MRSA detected, positive for mecA. |
| + | - | - | +/- | Valid | MRSA not detected. |
| - | + | - | +/- | Valid | MRSA not detected. |
| - | - | + | +/- | Valid | MRSA not detected. |
| - | - | - | + | Valid | MRSA not detected. |
| - | - | - | - | Invalid | The test result can not be evaluated. |



Important notes:

All samples of biological origin and used plates/swabs are to be treated as potential carriers of infectious diseases.

When working with chemicals or when handling samples of biological origin, the safety precautions of the laboratory must be observed.

Storage: -25°C -18°C **Usage:** +2°C +8°C



Before performing this test, read the instructions for use to familiarise yourself with the testing procedure.

You can find them on <https://frizbiochem.de/downloads/>

If you have any questions or problems, please contact service at FRIZ Biochem GmbH (<https://frizbiochem.de/get-in-touch/>).

REF FBC111

IVD