Primerdesign™ Ltd

MRSA: MecA and FemB

genesig®PLEX Kit

150 tests

GENESIG

Kits by Primerdesign

For general laboratory and research use only

Introduction to Methicillin-resistant *Staphylococcus aureus* (MRSA)

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a specific strain of the *Staphylococcus aureus* bacterium that has developed antibiotic resistance to all penicillin's, including methicillin and other narrow-spectrum β-lactamase-resistant penicillin antibiotics.

The resistant strain, MRSA was first discovered in the UK in 1961 and is now widespread, particularly in the hospital setting where it is commonly termed a superbug. MRSA may also be known as oxacillin-resistant *Staphylococcus aureus* (ORSA) and multiple-resistant *Staphylococcus aureus*, while non-methicillin resistant strains of *S. aureus* are sometimes called methicillin-susceptible *Staphylococcus aureus* (MSSA) if an explicit distinction must be made.

Although MRSA has traditionally been seen as a hospital-associated infection, community acquired MRSA strains have appeared in recent years, notably in the US and Australia. The abbreviations CA-MRSA (community-associated MRSA) and HA-MRSA (hospital-associated MRSA) are now commonly seen in medical literature.

Methicillin resistance arises by acquisition of a *staphylococcal* cassette chromosome, SCCmec, and is conferred by the mecA gene. Expression of this gene yields PBP2a, a penicillin binding protein with reduced affinity for β-lactam rings (the primary active-site of the β-lactam antibiotics). Some strains of *S. aureus* over-express β-lactamase and appear to be resistant to oxacillin and, rarely, methicillin despite being mecA-negative. They have slightly raised minimum inhibitory concentrations (MICs) and may thus be described as "minimally resistant".

Other strains express modified PBPs (not PBP2) and exhibit varying degrees of β-lactam antibiotic resistance. Not only are MRSA strains resistant to the usual antibiotics, but a curious interbreeding with community *staphylococcus* has led to additional concerns. Many MRSA isolates found outside of medical facilities (CA-MRSA), have acquired the Panton-Valentine leukocidin factor, a gene that produces a series of chemicals that make these MRSA particularly invasive as well as resistant.

Specificity

This genesigPLEX kit is designed for the *in vitro* detection of mecA and femB genes from MRSA (methicillin resistant *staphylococcus aureus*). The kit is designed to have the broadest detection profile possible while remaining specific to the MRSA genome.

Assays are designed to specifically detect the query target(s) at the >95% homology and identity level in silico and to prevent detection of any off-target sequences (unless specified).

The dynamics of genetic variation means that new sequence information may become available after the initial design. Primerdesign periodically reviews the detection profiles of our kits and when required releases new versions.

The mecA gene is a plasmid-based gene responsible for antibiotic resistance. FEMB is a chromosomal gene specific to *S. aureus*. A positive result for both markers has been validated as a reliable test for MRSA.

The primers and probe have 100% homology with all reference sequences in the NCBI data base including those listed below.

MecA sequence specificity: AM292304, AB266532, AB266531, AB245471, AB245470, AB236888, AY894415, DQ106887, AY786579, AY271717, AB221124, AB221123, AB221122, AB221121, AB221120, AB221119, BA000017, AP006716, CP000046, AM048803, AM048802, CP000255, AJ810121, AJ810120, AB047089, Y14051.1, Y13095.1, Y13096.1, Y00688.1, X52593.1, BA000033, BA000018, AB121219, CP000029, X52592.1, AB063172, AB033763, D86934.2, AB096217, AB063173, AB037671.

FEMB sequence specificity: CP000703.1, BA000017.4, CP000046.1, CP000253.1, CP000255.1, X17688.1, X571857.1, BA000033.2, BA000018.3, BX571856.1

If you require further information or have a specific question about the detection profile of this kit, then please send an email to enquiry@primerdesign.co.uk and our bioinformatics team will answer your question.

Kit contents

- **Multiplex primer/probe mix (150 reactions BROWN)**
  FAM, VIC and Cy5 labelled (see table below)

Target	Fluorophore
MecA	VIC
FemB	FAM
Internal extraction control	Cy5

- **MecA positive control template (RED)**
- **FemB positive control template (RED)**
- **Internal extraction control DNA (BLUE)**
- **Lyophilised oasig™ Standard Master Mix (SILVER)**
- **oasig™ resuspension buffer (BLUE)**
- **Template preparation buffer (YELLOW)**
  for resuspension of positive control template
- **RNase/DNase-free water (WHITE)**
  for resuspension of primer/probe mix

Reagents and equipment to be supplied by the user

Real-time PCR Instrument

DNA extraction kit
This kit is recommended for use with genesig EASY DNA/RNA Extraction kit. However, it is designed to work well with all processes that yield high quality DNA with minimal PCR inhibitors.

Pipettors and tips

Vortex and centrifuge

Thin walled 0.1 ml tubes

qPCR plates

Kit storage and stability

This kit is stable at room temperature but should be stored at -20°C on arrival. Once the lyophilised components have been resuspended, they should not be exposed to temperatures above -20°C for longer than 30 minutes at a time and unnecessary repeated freeze/thawing should be avoided. The kit is stable for six months from the date of resuspension under these circumstances. Primerdesign does not recommend using the kit after the expiry date stated on the pack.

Suitable sample material

All kinds of sample material suited for PCR amplification can be used. Please ensure the samples are suitable in terms of purity, concentration, and RNA/DNA integrity. Always run at least one negative control with the samples. To prepare a negative control, replace the template DNA sample with RNase/DNase-free water.

Dynamic range of test

Under optimal PCR conditions genesig kits have very high priming efficiencies of >90% and can detect between $1X10^8$ and $1X10^2$ copies of target template.

Notices and disclaimers

This product is developed, designed, and sold for research purposes only. It is not intended for human diagnostic or drug purposes or to be administered to humans unless clearly expressed for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. During the warranty period Primerdesign genesig detection kits allow precise and reproducible data recovery combined with excellent sensitivity. For data obtained by violation to the general GLP guidelines and the manufacturer's recommendations the right to claim under guarantee is expired. PCR is a proprietary technology covered by several US and foreign patents. These patents are owned by Roche Molecular Systems Inc. and have been sub-licensed by PE Corporation in certain fields. Depending on your specific application you may need a license from Roche or PE to practise PCR. Additional information on purchasing licenses to practise the PCR process may be obtained by contacting the Director of Licensing at Roche Molecular Systems, 1145 Atlantic Avenue, Alameda, CA 94501 or Applied Biosystems business group of the Applera Corporation, 850 Lincoln Centre Drive, Foster City, CA 94404. In addition, the 5' nuclease assay and other homogeneous amplification methods used in connection with the PCR process may be covered by U.S. Patents 5,210,015 and 5,487,972, owned by Roche Molecular Systems, Inc, and by U.S. Patent 5,538,848, owned by The Perkin-Elmer Corporation.

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Principles of the test

Real-time PCR
Individual primer and probes designed for each gene have been combined into a single reaction and these can be detected through the different fluorescent channels as described in the kit contents.

The primer and probe mix provided exploits the so-called TaqMan® principle. During PCR amplification, forward and reverse primers hybridise to the target cDNA. Fluorogenic probes are included in the same reaction mixture which consists of a DNA probe labelled with a 5`-dye and a 3`-quencher. During PCR amplification, the probe is cleaved, and the reporter dye and quencher are separated. The resulting increase in fluorescence can be detected on a range of qPCR platforms.

Positive control
For a positive control, the kit contains two positive controls, one with a template for MecA and one with a template for FemB. The MecA positive control will give amplification through the VIC channel and the FemB positive control will give amplification through the FAM channel. Each time the kit is used, at least one positive control reaction for each gene must be included in the run. A positive result indicates that the primers and probes for detecting each gene are working properly in that particular run. If a negative result is obtained the test results are invalid and must be repeated. Care should be taken to ensure that the positive controls do not contaminate any other kit component which would lead to false positive results. This can be achieved by handling these components in a post PCR environment. Care should also be taken to avoid cross-contamination of other samples when adding the positive control to the run. This can be avoided by sealing all other samples and negative controls before pipetting the positive controls into the positive control wells.

Negative control
To confirm the absence of contamination, a negative control reaction should be included every time the kit is used. For this reaction, the RNase/DNase-free water should be used instead of template. A negative result indicates that the reagents have not become contaminated while setting up the run.

Internal DNA extraction control
When performing DNA extraction, it is often advantageous to have an exogenous source of DNA template that is spiked into the lysis buffer. This control DNA is then co-purified with the sample DNA and can be detected as a positive control for the extraction process. Successful co-purification and qPCR amplification of the control DNA also indicates that PCR inhibitors are not present at a high concentration. The primers and probe necessary to detect the internal extraction control are included in the multiplex primer and probe mix. The amplification of the internal control does not affect the sensitivity of the test and is detected separately through the Cy5 channel. The Internal control will give a Cq value of 28+/-3, but this can vary greatly depending on the efficiency of sample extraction and level of sample dilution.

Resuspension protocol

To minimise the risk of contamination with foreign DNA, we recommend that all pipetting be performed in a PCR clean environment. Ideally this would be a designated PCR lab or PCR cabinet. Filter tips are recommended for all pipetting steps.

1. **Pulse-spin each tube in a centrifuge before opening.**
   This will ensure lyophilised primer and probe mix is in the base of the tube and is not spilt upon opening the tube.

2. **Resuspend the primer/probe mixes in the RNase/DNase-free water supplied, according to the table below:**
   To ensure complete resuspension, vortex the tube thoroughly.

Component – resuspend in water	Volume
Multiplex primer/probe mix (**BROWN**)	165 µl

3. **Resuspend the internal control template and positive control templates in the template preparation buffer supplied, according to the table below:**
   To ensure complete resuspension, vortex the tube thoroughly.

Component – resuspend in template preparation buffer	Volume
Internal extraction control DNA (**BLUE**)	600 µl
MecA positive control template (**RED**)*	500 µl
FemB positive control template (**RED**)*	500 µl

*** These components contain high copy number template and are a VERY significant contamination risk. They must be opened and handled in a separate laboratory environment, away from the other components.**

4. **The lyophilised oasig Standard Master Mix in oasig resuspension buffer, according to the table below:**

Component – resuspend in oasig resuspension buffer	Volume
Lyophilised oasig Standard Master Mix (SILVER)	525 µl

DNA extraction

The internal extraction control DNA can be added to either the DNA lysis/extraction buffer or to the DNA sample once it has been resuspended in lysis buffer.

DO NOT add the internal extraction control DNA directly to the unprocessed biological sample as this will lead to degradation and a loss in signal.

1. **Add 4 µl of the Internal extraction DNA (BLUE) to each sample in DNA lysis/extraction buffer.**

2. **Complete the DNA extraction according to the manufacturer's recommended protocols.**

qPCR detection protocol

1. **For each DNA sample prepare a reaction mix according to the table below:**
   Include sufficient reactions for positive and negative controls.

Component	Volume
Oasig Standard Master Mix (SILVER)	10 µl
Multiplex primer/probe mix (**BROWN**)	1 µl
RNase/DNase-free water (WHITE)	4 µl
Final volume	**15 µl**

2. **Pipette 15 µl of this mix into each well according to your qPCR experimental plate set up.**

3. **Pipette 5 µl of DNA sample into each well according to your experimental plate set up.**
   For negative control wells use 5 µl of RNase/DNase-free water. The final volume in each well is 20 µl.

4. **Pipette 5 µl of positive control template into each positive control well according to your plate set up.**
   This will require at least one well each for MecA and for FemB. The final volume in each well is 20 µl.

qPCR amplification protocol

Amplification conditions using oasig Standard Master Mix

	Step	Time	Temp
Cycling x 50	Enzyme activation	2 mins	95°C
	Denaturation	10 secs	95°C
	DATA COLLECTION*	60 secs	60°C

* Fluorogenic data should be collected during this step through the FAM, VIC and Cy5 channels.

Interpretation of results

Positive control
The MecA positive control well should give an amplification plot through the VIC channel and the FemB positive control well should give an amplification plot through the FAM channel. There is no internal control template within either of the positive controls so the Cy5 channel should give no signal (flat amplification plot). The positive control signals indicate that the kit is working correctly to detect each gene.

No template control (NTC)
The NTC should give a flat line (flat amplification plots) through all channels. Signals in the NTC indicate cross contamination during plate set up.

Internal PCR control
The Cq value obtained with the internal control will vary significantly depending on the extraction efficiency, the quantity of DNA added to the PCR reaction and the individual machine settings. Cq values of 28±3 is within the normal range. When amplifying an MRSA sample with a high genome copy number of mecA and/or femB, the internal extraction control may not produce an amplification plot. This does not invalidate the test and should be interpreted as a positive experimental result.

Sample data
Presence of the genes are detected in the channels indicated in the kit contents section. Positive signals indicate positive tests for these genes. Samples may contain both genes, therefore positive results in the FAM and VIC channels may be present.

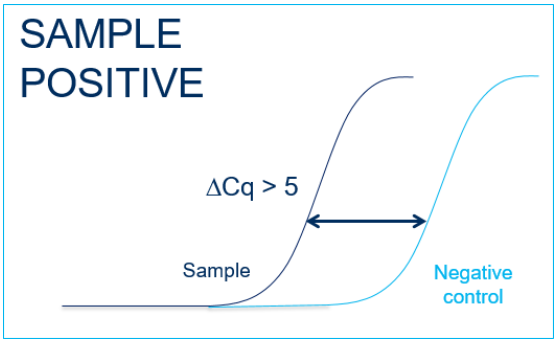
Summary of data interpretation

Target (FAM/VIC)	Internal Extraction Control (Cy5)	Positive Controls	Negative Control	Interpretation
VIC +	**+ / -**	**+**	**-**	MecA POSITIVE RESULT
FAM +	**+ / -**	**+**	**-**	FemB POSITIVE RESULT
-	**+**	**+**	**-**	NEGATIVE RESULT

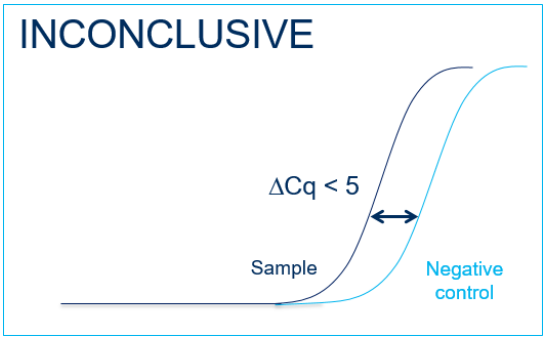
+ / -	**+ / -**	**+**	**≤35**	EXPERIMENT FAILED Due to test contamination
+ / -	**+ / -**	**+**	**>35**	*
-	**-**	**+**	**-**	SAMPLE PREPARATION FAILED
+ / -	**+ / -**	**-**	**+ / -**	EXPERIMENT FAILED

Positive control template (RED) is expected to amplify between Cq 16 and 23. Failure to satisfy this quality control criterion is a strong indication that the experiment has been compromised.

* Where the test sample is positive, and the negative control is positive with a Cq >35, the sample must be reinterpreted based on the relative signal strength of the two results:



If the sample amplifies > 5 Cq earlier than the negative control, then the sample should be reinterpreted (via the table above) with the negative control verified as negative.



If the sample amplifies < 5 Cq earlier than the negative control, then the positive sample result is invalidated, and the result should be determined inconclusive due to test contamination. The test for this sample should be repeated.