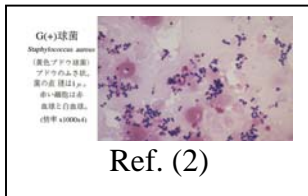


Staphylococcus aureus MecA Detection with real time PCR reagents

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Overview:



Ref. (2)

Staphylococcus aureus remains one of the most common and serious bacterial infections. Resistance to methicillin has become a common feature of this organism. The presence of the mecA gene identifies this resistance feature and detection of the FemA_SA sequence identifies the organism as S. aureus.

Primers and probes are available for amplification and detection of the S. aureus MecA and FemA_SA genes.. The MecA primers are specific for a 93 bp sequence. The FemA_SA primers amplify an 83 bp sequence. The MecA and FemA_SA detection probes are molecular beacons. (1)

Products

Products	Catalog No.	Quantity
S. aureus MecA FAM-BHQ1 primers-probe <i>Store at -20C. 25-20 µl reactions</i> <i>Typical use: Add 2X master mix and DNA, then thermal cycle.</i> <i>Attostar reagent contains primers and probe. Detect at 510nm.</i>	PP2200	0.055ml
AttoMaster 2X Mix for qPCR <i>Store at -20°C. 125-20 µl reactions</i> <i>Contains Taq polymerase (requires heat activation), dNTPs (0.4 mM) with optimal dUTP to dTTP ratio, heat labile UDG, Mg(6 mM), and buffer.</i> <i>Typical use: Add Attostar Primer-probe, DNA, and then thermal cycle.</i>	AM10	1.25 ml
S. aureus MecA Plasmid 200 pg/ml <i>Store at -20C.</i> <i>Typical use: make serial 10 fold dilutions in TE for standard curve, diluting 5 µl into 45 µl TE buffer.</i>	PLAS2200	0.25ml

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Detection of *S. aureus* MecA DNA:

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Thermal cycle conditions for PCR reactions on RotorGene*

*Similar cycle conditions and reaction volumes may be used on many other thermal cyclers.

- 25°C 10 min (UDG treatment time)
- 95°C 120 sec (activation for AttoMaster polymerase)
- 40 cycles
 - 95°C 15 seconds
 - 60°C 30 seconds RotorGene Channel Setup FAM/Sybr, Cy5; Gain 7
 - 72°C 30 seconds

FAM/Sybr has a source of 470nm and Detector 510nm (LightCycler use F1)
Cy5 has a source of 625nm and Detector 660hp nm
Quasar 670 has the same fluorescent absorption and emission as Cy5.

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Thermal cycle conditions for PCR reactions on LightCycler

- 25°C 10 min (UDG treatment time)
- 95°C 120 sec (activation for AttoMaster polymerase)
- 40 cycles
 - 95°C 15 seconds
 - 60°C 30 seconds acquire fluorescent signal on F1 gain =1
 - 72°C 30 seconds
 - 40°C 30 seconds cool

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Use of the *S. aureus* MecA plasmid:

Dilute the plasmid in TE to prepare a standard curve. Common dilutions would be 10-fold from 200 to 0.002pg/ml. The 0.02 pg/ml plasmid dilution contains 10 copies of plasmid in 2 µl.

.....
Attostar Primers/probe PP2200 RotorGene-PCR 20µl reactions

RotorGene Reagents needed for 20 ul PCR final reaction tube volumes										
Reaction tube number	1	2	3	4	5	6	7	8	9	10
Attostar Primer-Probe FAM labeled (10X)	2	4	6	8	10	12	14	16	18	20 µl
Master mix (2X)	10	20	30	40	50	60	70	80	90	100 µl
Dispense 12 ul / reaction tube										
Add 8 ul DNA / reaction tube										

Attostar Primers/probe PP2200 LightCycler-PCR 20 µl reactions

LightCycler Reagents needed for 20 ul PCR final reaction tube volumes										
Reaction tube number	1	2	3	4	5	6	7	8	9	10
Attostar Primer-Probe (10X)	2	4	6	8	10	12	14	16	18	20 µl
Master mix (2X)	10	20	30	40	50	60	70	80	90	100 µl
BSA 1 mg/ml	1	2	3	4	5	6	7	8	9	10 µl
Dispense 13 ul / reaction tube										
Add 7 ul DNA / reaction tube										

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Extraction / amplification control with T4 bacteriophage

Adding T4 bacteriophage (BAC130) to the sample provides DNA for extraction, amplification, and reaction condition PCR controls.

When added to a sample, T4 adds a known amount of DNA. The T4 DNA can then be extracted, amplified, and detected as a control. T4 controls for the efficiency of DNA extraction, the presence of PCR amplification inhibitors, intact amplification reagents (DNA polymerase, buffer, dNTPs), and instrument function (thermal cycling and fluorescent detection system).

The T4 DNA may be detected in a separate PCR reaction (using FAM labeled T4 probe in PP100). Or the T4 DNA and test organism DNA may be detected using a multiplex reaction (using Quasar 670 labeled T4 probe in PP160 and FAM labeled test organism probe).

Brief procedure for use of T4 as extraction and amplification control:

- Add 5µl T4 bacteriophage to the sample. Proceed with DNA extraction. Dilutions of the bacteriophage may be made to give a final PCR Ct value that is about 35. At this dilution, the phage is more sensitive, i.e. more likely, to detect a poor extraction or the presence of PCR inhibitors in the reaction.

Please refer to the BAC130, PP100, and PP160 product literature.

T4 multiplex reaction (PP160)

RotorGene Multiplex PCR 20µl reactions

Multiplex Reagents needed for 20 ul PCR final reaction tube volumes											
Reaction tube number	1	2	3	4	5	6	7	8	9	10	
Attostar Primer-Probe PP160	2	4	6	8	10	12	14	16	18	20	µl
Primer-Probe mix for TEST organism	2	4	6	8	10	12	14	16	18	20	µl
2 x master mix	10	20	30	40	50	60	70	80	90	100	µl
Dispense 14 ul / reaction tube											
Add 6 ul DNA / reaction tube											

Reaction tube number	11	12	13	14	15	16	17	18	19	20	
Attostar Primer-Probe PP160	22	24	26	28	30	32	34	36	38	40	µl
Primer-Probe mix for TEST organism	22	24	26	28	30	32	34	36	38	40	µl
2 x master mix	110	120	130	140	150	160	170	180	190	200	µl
Dispense 14 ul / reaction tube											
Add 6 ul DNA / reaction tube											

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Reference:

- 1) <http://www.molecular-beacons.org/Introduction.html>
- 2) Image: *S. aureus*:
<http://www.buddycom.com/bacteria/gpc.html>

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Enlarged image:

S. aureus gram positive cocci in pairs, chains and clusters. (2)

