

mcrA Methanocella

QPCR mixture and conditions

Name	Sequence	Target	Reference
mlas-mod F	GGY GGT GTM GGD TTC ACM CAR TA		Modified from: Steinberg and Regan AEM 2008
mcrA-rev R	CGT TCA TBG CGT AGT TVG GRT AGT		Steinberg and Regan AEM 2008
mcell P	CTR GGC TTC TWC GGS TAC GAY CT		Self designed

Reagent	Final conc.	1 tube (25 µL)	Plate (25 µL × 100)
PCR H ₂ O		4.2	420
ReadyMix (Sigma)	1X	12.5	1250
MgCl ₂ (25 mM)	3.5 mM	2*	200*
BSA (50 µg/µL)	0.8 µg/µL	0.4	40
FITC (1:1000)		0.25	25
mlas F (25 µM)	0.25 µM	0.25	25
mcrA-rev R (25 µM)	0.25 µM	0.25	25
mcell P[†] (25 µM)	0.15 µM	0.15	15
Template		5	5 × 100

*Buffer contains 1.5mM of MgCl₂ at final conc.

[†] Probe must be dual-labelled either with 5'-6-FAM, 3'-BHQ1 or any other valid combination.

Programme:

1. 95 °C – 5'
 X40 {
2. 95 °C – 30"
3. 57 °C – 45"
4. 62 °C – 30" – take snapshot}