

mcrA general

qPCR mixture and conditions

Name	Sequence	Target	Reference
mlas-mod	GGY GGT GTM GGD TTC ACM CAR TA		Modified from: Steinberg and Regan AEM 2008
mcrA-rev	CGT TCA TBG CGT AGT TVG GRT AGT		Steinberg and Regan AEM 2008

Reagent	Final conc.	1 tube (25 µL)	Plate (25 µL × 100)
PCR H ₂ O		2.85	285
ReadyMix (Sigma)	1X	12.5	1250
MgCl ₂ (25 mM)	3.5 mM	3.5	350
BSA (50 µg/µL)	0.8 µg/µL	0.4	40
FITC (1:1000)		0.25	25
mlas-mod (25 µM)	0.25 µM	0.25	25
mcrA-rev (25 µM)	0.25 µM	0.25	25
Template		5	5 × 100

Programme:

1. 94 °C – 5'
- X40 {**
2. 95 °C – 30"
3. 57 °C – 45"
4. 72 °C – 30"
5. 84 °C – 10" – take snapshot}
6. **Melting curve:** 75 °C – 94.8 °C – 6". Increase by 0.2 °C