SOP: M013

Mycobactin HR Media and Plates

Materials

MilliQ water	1 L disposable filter (Nalgene)
$MgCl_2$	Magnetic stir bar and plate
10 M NaOH	2 x 2 L bottles
L-asparagine	UltraPure agarose (Invitrogen)
2 M H_2SO_4	Large culture plates (15 ml)
$ZnSO_4$	Class II Biosafety hood
$MnCl_2$	Ziplock bags
KH_2PO_4	100 ml bottle

Protocol

1._____Place 950 ml MilliQ water in 2 L bottle reserved for mycobactin work. Add 20 ml glycerol and stir until homogenous.

2._____Adjust pH to 7.5 with concentrated NaOH, then add 5 g L-asparagine, 5 g KH_2PO_4.

3._____Adjust pH to 6.8 with H_2SO_4 and add Zn^{2+}, Mn^{2+}, and Mg^{2+} to obtain final concentrations shown below (Note 1).

$ZnSO_4$	0.46 µg/ml
$MnCl_2$	0.10 µg/ml
$MgCl_2$	0.04 **mg**/ml

4._____Allow solution to stir at least 30 min, filter into disposable container, then transfer to fresh 2 L bottle.

5._____Transfer 80 ml media to 100 ml bottle for autoclaving. This solution will be transported to BSL-3 facility for growth of Mtb.

6._____Add 18.4 g UltraPure agarose to 920 ml media in 2 L bottle. Autoclave at least 30 min.

7._____Working in Class II BSC, transfer warm media to large plates, 125 ml each. Once dry, transfer plates to ziplock bags for storage at 4°C.

Notes

(1) This should make the ions more soluble prior to autoclaving media. Since these are such minute quantities, it is more efficient to aliquot from stock solutions made in endotoxin-free H_2O.

References

RM Hall and C Ratledge (1982) A simple method for the production of mycobactin, the lipid-soluble siderophore from mycobacteria FEMS Microbiology Letters 15: 133-136.