

SOP: PP014**Preparation of Mycolic acid methyl esters****Materials and Reagents:**

1. Purified mAGP, 50 to 150 mg
2. Tetrabutyl ammonium hydroxide (TBAH) (Fluka 86854), 15% solution
3. Dichloromethane (Aldrich 270997-100ml)
4. Iodomethane (Aldrich 289566-100g)
5. Diethyl ether (VWR BJ107-1)
6. Glass tubes with PTFE-lined lids, 13 x 100 mm
7. Glass pipet, 5 ml
8. Pipet bulb, rubber
9. Heat block, 100°C
10. Glass capillary pipet, 100 µl
11. Glass capillary pipettor, 100 µl
12. Platform rocker
13. Benchtop centrifuge
14. Pasteur pipets, glass
15. Pasteur pipet bulb, rubber
16. N₂ bath
17. Vortex
18. Pan sonicator
19. Mettler-Toledo balance
20. Materials and equipment for TLC
21. Petroleum ether (VWR BJ317-1)

Protocol:

- 1._____ Transfer mAGP into a 13 x 100 mm glass tube.
- 2._____ Add 2 ml of 15% TBAH solution (note 1).
- 3._____ Cap tube and place in heat block at 100°C for two hours (note 2).
- 4._____ Remove from heat block and let cool to room temperature.
- 5._____ Add 2 ml of dichloromethane.
- 6._____ Add 100 µl of iodomethane (note 3).
- 7._____ Cap tube and place on platform rocker at room temperature for one hour.
- 8._____ Centrifuge at 2,500 x g, 4°C for 10 minutes.
- 9._____ Transfer organic, lower layer to a 13 x 100 mm glass tube (note 4). The insoluble pellet should be saved for further purification (note 5).
- 10._____ Completely dry organic layer under a stream of nitrogen (note 6).
- 11._____ Add 3 ml of diethyl ether, cap tube and re-suspend dried organic layer (note 7).
- 12._____ Transfer to a new, pre-weighed 13 x 100 mm glass tube
- 13._____ Completely dry under a stream of nitrogen and weigh.
- 14._____ Re-suspend in a minimal amount of dichloromethane.
- 15._____ Load 10 to 20 µg onto a 10 x 10 cm aluminum-backed TLC plate (note 8).

16. _____ Run the plate six times in solvent system petroleum ether-diethyl ether (95:5), allowing the plate to dry between runs.
17. _____ Visualize purified MAME with charring spray (note 9).

Notes:

1. All organic and acidic compounds need to be transferred using glass pipets or glass capillary pipets.
2. Two hours is the minimum time for hydrolysis, but the reaction may be run overnight.
3. Iodomethane acts as a catalyst for phase transfer to make methyl esters.
4. It is important not to contaminate the organic layer with debris from the aqueous layer. To do this, expel several drops of air while passing the Pasteur pipet through the upper layer until the tip is in the lower layer.
5. The insoluble material is de-mycolated AGP, and should be saved and used for the purification of arabinogalactan and peptidoglycan (SOP PP012 and PP013). Using a glass Pasteur pipet, remove the aqueous layer and dry the pellet in a chemical fume hood.
6. See SOP SP031 for use of the N₂ Bath. A white, salty precipitate should be remaining in the tube.
7. The pellet is difficult to re-suspend, and will need to be pan sonicated and vortexed vigorously. This step removes contaminating salts from the MAME.
8. See SOP SP033 for running TLC.
9. See SOP R011 for preparation of charring spray.

Reference:

Slayden R. A., C. E. Barry III. Analysis of the Lipids of *Mycobacterium tuberculosis*. *Methods in Molecular Medicine, Mycobacterium tuberculosis Protocols*. T. Parish and N. G. Stoker, ed. 2001. 229-246.

Personal correspondence with Dr. Richard A. Slayden, Mycobacterial Research Laboratories, CSU, Fort Collins, CO.