SOP: PP018.1
Updated 1/31/13

Isolation of Total Lipid

Materials and Reagents:

1. *M. tuberculosis* γ-irradiated whole cells, 50 to 200 g (wet weight)
2. Lyophilizer
3. Erlenmeyer flask, 1800 ml
4. Chloroform, HPLC-grade (VWR BJ049-1)
5. Methanol, HPLC-grade (VWR BJ230-1)
6. Magnetic stir bar, large
7. Parafilm
8. Magnetic stir plate
9. Reach-in incubator, 55°C
10. Whatman filter paper, 17 CHR
11. Buchner funnel
12. Graduated cylinder, 1000 ml
13. Separatory funnel, 2000 ml
14. Water, HPLC-grade (VWR BJ 365-4)
15. Round bottom flask, 1000 ml
16. Rotovap
17. Silica TLC plate, aluminum backed
18. Glass TLC tank, small
19. Capillary pipet, 10 µl
20. Capillary pipettor, 10 µl
21. Glass tubes with PTFE-lined lids, 13 x 100 mm
22. Capillary pipet, 100 µl
23. Capillary pipettor, 100 µl
24. N_2 bath

Protocol:

1._____ Lyophilize the *M. tuberculosis* γ-irradiated whole cells (note 1).

2._____ When completely dry, remove cells from the lyophilizer and weigh.

3._____ Transfer cells to a 1 or 2 L bottle.

4._____ Suspend cells in chloroform-methanol (2:1) at 30 ml/g of cells (notes 2 and 3).

5._____ Add a magnetic stir bar, cap the bottle tightly, and stir on magnetic stir plate overnight in a chemical fume hood.

6._____ Fold a round piece of filter paper into a cone, fit into a glass funnel, and secure above a clean bottle.

7._____ Pour the overnight extract slowly through the filter. Swirl the bottle with the cells once in a while.

8._____ Scrape cells from filter paper with a spatula, and return them to the original bottle. Stir with ½ the volume of the original 2:1 volume used, for 3-4 hours. Add this extract to the original overnight extract.

9._____ Repeat steps 7-8 for a total of 3 extractions, combining all extracts into one bottle.

10._____ Let de-lipidated cells air dry in the chemical fume hood. Save and store when dry, noting the cell lot# and date of extraction.

11._____ Measure total volume of filtered extract in a 1 L graduated cylinder.

12._____ Transfer organic supernatant into the 2 L separatory funnel.

13._____ Add the appropriate volume of water to make a final ratio of choloform/methanol/water 4:2:1 (note 4).

14._____ Cap funnel tightly, invert several times to mix thoroughly, and let sit on ring stand until aqueous and organic layers partition (note 5).

15._____ Collect lower organic layer into a pre-weighed 1 L round-bottom flask.

16._____ Dry the organic layer on the rotovap and weigh the extracted material (note 6).

17._____ Re-suspend lipid in chloroform-methanol (2:1) at a concentration of 20 mg per ml.

18._____ For quality control, aliquot 100 µg of lipid onto two 10 x 10 cm aluminum-backed TLC plates (note 7).

19._____ Run TLC in solvent system 65/25/4 chloroform/methanol/water and develop one plate with charring spray, the other with α-naphthol (note 8).

20._____ Aliquot 1 ml Folch-washed lipid into a tared glass tube, then dry to determine weight. Save this to be used as a control for later.

21._____ Aliquot total lipid into 13 x 100 mm glass tubes (default quantity 5 mg), and dry under a stream of nitrogen (notes 9 and 10).

22._____ Excess total lipid may be used to purify TDM, TMM, SL, PDIM, etc.

Notes:

1. See SOP SP004 for use of the lyophilizer.
2. Always use HPLC-grade or better solvents.
3. All use of organic solvents and filtering should take place in a chemical fume hood.
4. To calculate the amount of water to add, take the total volume of methanol added and divide in half. This is known as a Folch wash, and will cause highly polar lipids and oligosaccharides to crash out in a foamy layer.
5. This takes approximately 30 to 60 minutes.
6. See SOP SP037 for operation of the rotovap.
7. Include a similar amount of an older lot of total lipid for positive control. The two TLC sheets should be duplicates of one another.
8. See SOP SP033 for running and charring TLC, and SOP R011/012 for making of the detection solvents.
9. Transfer material using a 100 µl glass capillary pipet or very carefully with glass 5-10 ml pipets and rubber bulb.
10. See SOP SP031 for use of the N_2 bath.

Reference:

Minnikin D. E. In Bacterial Cell Surface Techniques (I. C. Hancock and I. R. Paxton, eds.) John Wiley & Sons, New York. Pp 125-135, 1988.