SOP: PP018.2
Updated 11/17/21

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
5. Methanol, HPLC-grade
6. Magnetic stir bar, large
7. Magnetic stir plate
8. Filter paper 413 (VWR, Cat 28310-172)
9. Graduated cylinder, 1000 ml
10. Separatory funnel
11. Water, HPLC-grade
12. Round bottom flasks
13. Rotovap
14. Silica TLC plate, aluminum backed
15. Glass TLC tank, small
16. Capillary pipet, 10 µl
17. Capillary pipettor, 10 µl
18. Glass tubes with PTFE-lined lids, 13 x 100 mm
19. Capillary pipet, 100 µl
20. Capillary pipettor, 100 µl
21. 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 500 mL or 1 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._____ Allow cells to settle at bottom of bottle before pouring through the filter.

8._____ Pour the overnight extract slowly through the filter (note 4).

9._____ Scrape cells from filter paper with a spatula and return them to the original bottle, using a glass funnel to aid in transfer. Stir with the volume of the original 2:1 volume used, for 3-4 hours. Add this extract to the original overnight extract (note 5).

10._____ Repeat steps 6-9 for a total of 3 extractions, combining all extracts into one bottle (note 6).

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

12._____ Measure total volume of filtered extract in a 1 L graduated cylinder (note 7).

13._____ Transfer organic supernatant into a clean glass bottle.

14._____ Add the appropriate volume of water to make a final ratio of chloroform/methanol/water 4:2:1. Add slowly in 10 mL increments while swirling the bottle.

15._____ Swirl suspension and transfer to a separatory funnel fitted into a ring stand until aqueous and organic layers partition (note 8).

16._____ Collect lower organic layer into a new bottle.

17._____ Transfer to a graduated cylinder to record volume.

18._____ Estimate total volume by aliquoting 0.5 ml to tared vial, drying under N2, and calculate based on final weight.

19._____ Dry the organic layer via rotary evaporation (note 9).

20._____ Re-suspend lipid in chloroform-methanol (2:1) at a concentration of 10 mg per ml.

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

22._____ Run TLC in solvent system 65/25/4 chloroform/methanol/water and develop one plate with CuSO4 charring spray, the other with α-naphthol (note 11).

23._____ If storing as a BEI product, aliquot total lipid into the requisite number of 13 x 100 mm glass tubes (default quantity 5 mg), and dry under a stream of nitrogen (notes 12 and 13).

24._____ 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. Allow a small volume of 2:1 to remain with the bulk of the cells at the bottom of the bottle, along with the stir bar. After the 3rd and final extraction, all cells will be transferred to filter paper for overnight drying.
5. Cells may be allowed to remain wet to allow for efficient scooping into the original bottle. Once dried, the filter may contain cell residue that can be scraped and transferred as well. Also, for the 3 rounds of extraction, the 1st can be a 3-4 hour, followed by an overnight, or vice versa (overnight extraction cannot be the last extraction). Rinse cells off of glass funnel into the bottle using 10-20 mL of 2:1 from next volume to be added.
6. While the 3rd extraction is filtering, take the liquid accumulated from the first 2 extractions and dry via Rotovap. The dry lipid will be re-suspended with the 3rd extract.
7. Add 2:1 to reach a total volume that is divisible by 6. To calculate the amount of water to add, take the total volume of filtered extract and divide by 6. This is known as a Folch wash and will cause highly polar lipids and oligosaccharides to crash out in a foamy layer.
8. Allow this to proceed overnight with a flask underneath the funnel in case of any leakage.
9. See SOP SP037 for operation of the rotovap.
10. Include a similar amount of an older lot of total lipid for positive control. The two TLC sheets should be duplicates of one another.
11. See SOP SP033 for running and charring TLC, and SOP R011/012 for making of the detection solvents.
12. Transfer material using a 100 µl glass capillary pipet or very carefully with glass 5-10 ml pipets and rubber bulb.
13. 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.