

SOP: PP018**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 μ l
20. Capillary pipettor, 10 μ l
21. Glass tubes with PTFE-lined lids, 13 x 100 mm
22. Capillary pipet, 100 μ l
23. Capillary pipettor, 100 μ l
24. N₂ 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 the 1800 ml Erlenmeyer flask.
4. ____ Suspend cells in chloroform-methanol (2:1) at 30 ml/g of cells (notes 2 and 3).
5. ____ Add a magnetic stir bar, and cover mouth of flask with parafilm.
6. ____ Place on magnetic stir plate in 55°C incubator and stir overnight.
7. ____ Remove from incubator and let cool to room temperature.
8. ____ Cut a round piece of filter paper and fold into a funnel shape; place inside the Buchner funnel.
9. ____ Filter extracted material through the funnel and collect the eluent in a 1000 ml graduated cylinder.
10. ____ Let de-lipidated cells air dry in the chemical fume hood; save and store when dry.
11. ____ Note volume of filtered eluent in the graduated cylinder.
12. ____ Transfer organic supernatant into the 2L separatory funnel.

13. ____ Add the appropriate volume of water to make a final ratio of chloroform-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 1000 ml 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, spot two 10 x 10 cm aluminum-backed TLC plates each with 15 mg of lipid (note 7).
19. ____ Run TLC in solvent system chloroform-methanol-water (65:25:4) and develop one plate with charring spray and one plate with α -naphthol spray (note 8).
20. ____ Aliquot remaining material into 13 x 100 mm glass tubes (default quantity provided is 5 mg), and dry under a stream of nitrogen (notes 9 and 10).

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 volume of methanol added and divide in half.
5. This takes approximately 30 to 60 minutes.
6. See SOP SP037 for operation of the rotovap.
7. See SOP SP033 for running TLC.
8. See SOP R011 and SOP R012.
9. Transfer material using a 100 μ l glass capillary pipet and capillary pipettor.
10. See SOP SP031 for use of the N₂ 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.