

SOP: PP036.1
Updated 1/31/13

Preparation of Acetone-Soluble and Insoluble Total Lipid

Materials and Reagents:

1. Chloroform (note 1)
2. Methanol
3. Acetone
4. Water
5. H37Rv total lipid (note 2)
6. Copper sulfate
7. Reagent scale accurate to 0.1 mg
8. Capillary pipettor 10 μ l
9. Silica gel 60 TLC sheets, aluminum backed
10. Water bath sonicator
11. Pipets, serological and Pasteur, glass
12. Teflon centrifugation tubes
13. 13 x 100 mm glass tubes, screw top (10 ml)
14. PTFE-lined caps for tubes
15. Chemical fume hood
16. Reagent sprayer
17. Heat gun
18. Round bottom flask (100-250 ml)
19. Rotary evaporation apparatus
20. Air bath
21. Ultracentrifuge
22. Small TLC tank

Protocol:

1. ____ Obtain 10.0 mg total lipid from H37Rv cells.
2. ____ Resuspend in 2.0 ml 2:1 (CHCl₃/CH₃OH) with aid of water bath sonication.
3. ____ Add resuspension to cold acetone dropwise, and allow trituration to proceed overnight at -20°C (note 3).
4. ____ Transfer solution to teflon centrifuge tubes and spin at 20,000 x g at 4°C for 30 minutes.
5. ____ Transfer supernatant to tared round bottom flask labeled acetone-soluble lipid, and dry by rotary evaporation.
6. ____ Resuspend in 2-3 ml 2:1 and transfer to appropriate tared glass tube.
7. ____ Dry in air bath with nitrogen at room temperature. Measure weight of dried lipid.
8. ____ Resuspend pellet from centrifugation in 2-3 ml 2:1 and transfer to tared tube for acetone-insoluble lipid.
9. ____ Dry in air bath and measure weight of dried acetone-insoluble lipid.
10. ____ Run 25 μ g each sample on duplicate 10 x 10 cm TLC sheets with 65/25/4 CHCl₃/CH₃OH/H₂O (note 4).
11. ____ Stain one sheet with CuSO₄ and the other with α -naphthol, charring both with air gun (note 5).
12. ____ Aliquot both lipids in 13 x 100 mm tubes, 0.5 mg each, and store in the humidior.

Notes:

1. All reagents should be HPLC-grade.
2. See Isolation of Total Lipid SOP PP018.

3. Acetone volume in 45 ml Teflon tubes should be at least 10-fold that of resuspended total lipid, and should be equilibrated at -20°C overnight.
4. See Thin Layer Chromatography SOP SP033.
5. Fewer bands may be visualized near the top of the sheet with α -naphthol, as this stain will be specific for glycoside-containing lipids, which will be more polar.