

SOP: PP034**Preparation of Sulfolipid-1****Materials**

Lyophilized H37Rv cells (8-12 g)
Silica gel 60
CHCl₃ ACS grade
CHCl₃ B&J grade
CH₃OH B&J grade
16x 100 mm glass tubes + caps
Sterile sand
Trehalose dimycolate (TDM) standard
Sulfolipid-1 (SL-1) standard
Charring spray (CuSO₄)
250 ml glass column (Kontes)
Sep-Pak C18 columns (Waters, WAT023501)
Bottles, 1L, 500 ml
Stir plate
Chemical fume hood
Rotary evaporation equipment
Round-bottom flasks
10 ml glass syringe
Nitrogen bath
Small TLC tank (Kontes)
Hot air gun
Spray apparatus

Protocol

1. _____ Obtain total lipid from H37Rv by stirring lyophilized cells three separate times with CHCl₃/CH₃OH (2:1), collecting filtered extract in a large bottle. [Note 1]
2. _____ Use rotary evaporation to dry down total lipid to 20-40 ml.
3. _____ Use water bath sonication to ensure lipids are well suspended, adding more solvent if necessary. [Note 2]
4. _____ Rinse a glass column with 250 ml reservoir, and fitted with glass wool at the tip, with CHCl₃ in preparation for pouring the column. Mark the 20 ml line on the outside to use as a reference.
5. _____ Swirl 10-20 g silica gel 60 in a small beaker with CHCl₃, and slowly pour into glass column. Allow solvent to slowly drain and add more silica solution as the column begins to stack.
6. _____ Top off the column with sterile sand. [Note 3]
7. _____ Wash the column with five column volumes of ACS grade CHCl₃. Leave 1-2 ml solvent on top of column.
8. _____ Apply the total lipid via Pasteur pipet, near top of column. Once several milliliters have been aliquoted, switch to larger glass pipet to add rest of lipid. Collect the flowthrough as load. [Note 4]
9. _____ Rinse the round bottom flask with 10 ml 2:1 and similarly load onto column.
10. _____ Wash the column with 10 column volumes of ACS grade CHCl₃. As before, collect the fraction to check later by TLC.

11. ____ Elute with 10 column volumes of 5% CH₃OH in CHCl₃, then with 10% and 45% CH₃OH, collecting fractions separately.
12. ____ Dry down each fraction by rotary evaporation. Resuspend in 5-10 ml 2:1 CHCl₃/CH₃OH, using the same volume for each fraction.
13. ____ Check each fraction by TLC. Include TDM and SL-1 controls, and visualize the bands by charring. [Note 5]
14. ____ Divide the 5% CH₃OH resuspensions among several glass tubes, labeling them appropriately. Make 8-10 fold dilution with CH₃OH for each. [Note 6]
15. ____ Prepare 1 Sep-Pak cartridge per 4 tubes of lipid. Prep each column with 4 ml 95% acetonitrile, 5 ml CH₃OH, then 2 ml 80% CH₃OH. [Note 7]
16. ____ Load 4-5 ml of resuspended lipid onto a column at a time, using a slower flow rate than used during the column preparation steps.
17. ____ Wash each column with 4 ml CH₃OH and collect. Slowly elute with 6 ml 25% CHCl₃ in CH₃OH. [Note 8]
18. ____ Wash columns with 4 ml 40% CHCl₃ in CH₃OH, then elute with 6 ml 60% CHCl₃. [Note 9]
19. ____ QC the sulfolipid by running 2D TLC, loading SL alone and one with a control overlay.
20. ____ Perform NMR structure confirmation.

Notes

- (1) All organic solvents should be used in a chemical fume hood. Make sure to use glass pipets with rubber bulb for all work with solvents. Each extraction should be at least 4 hours, and one should be conducted overnight.
- (2) Make sure to keep the round-bottom flask capped with a glass stopper while sonicating. Carefully swirl the solvent and vent the flask frequently.
- (3) This will maintain the integrity of the top of the column as new solvents are added.
- (4) Collect large volumes of flowthrough in 500 ml bottles, pre-rinsed with similar solvent. All collections will be evaporated down to perform TLC checks.
- (5) Most of the TDM (upper band) and sulfolipid-1 (middle band) should elute in the 5% CH₃OH fraction. The band near the application line may be TMM.
- (6) This prepares the lipid for binding to reverse-phase C18 cartridges. The dried lipid will not solubilize well in methanol alone. Ensure that each aliquot is efficiently resuspended, with no apparent precipitation, to avoid product loss. Dilute no more than 1 ml resuspension per tube.
- (7) Use a 10 ml glass syringe to push solvents through. The samples can be loaded onto the columns with the same syringe.
- (8) This is about 20 column volumes. The lower band, or SL-1, should elute here.

(9) TDM is eluted at this percentage. Run a 1D TLC of the washes and the 25% and 60% CHCl₃ elutions along with TDM and SL-1 controls.