SOP: SP033

Thin-Layer Chromatography

Materials and Reagents:

1. Thin layer chromatography plates (note 1)
2. Organic solvents (note 2)
3. TLC tank (note 3)
4. Scissors or paper cutter
5. Foil
6. Ruler
7. Pencil
8. Capillary pipettor, 10 µl
9. Capillary pipets, glass, 10 µl
10. Plastic wrap
11. Lead weight
12. Chemical fume hood
13. TLC developer sprayer, 250 ml (Kontes 422530-0250)
14. TLC developer (note 4)
15. Heat gun, 200-300^{o}F
16. Scanner

Protocol:

1. _____ Using scissors or paper cutter, cut TLC plate to appropriate size (note 5).

2. _____ Using a ruler and pencil, draw a line 1 cm above and parallel to TLC plate bottom.

3. _____ Mark and label spots for application of compound to TLC plate (note 6).

4. _____ Using a 10 µl glass capillary tube in a 10 µl glass capillary pipettor, add compound(s) to TLC plate (note 7).

5. _____ Let all compounds applied to TLC plate dry completely.

6. _____ Thoroughly wash TLC tank (note 8).

7. _____ Make fresh TLC solvent and add to tank (note 9).

8. _____ Cover tank opening with plastic wrap, followed by tank lid, and place lead weigh on top. Allow tank to equilibrate for 5-10 minutes (note 10).

9. _____ Place plate(s) in equilibrated TLC tank.

10. _____ Let plate(s) sit in tank until solvent front reaches top of plate.

11. _____ Remove plate and let dry completely on foil in chemical fume hood.

12. _____ Place plate in TLC spraying area (note 11).

13. _____ Thoroughly spray TLC plate with desired developer in TLC sprayer attached to compressed air line in chemical fume hood.

14. _____ If necessary, heat TLC plate with heat gun to complete plate development.

15. _____ Immediately scan developed TLC plate to preserve results (note 12).

16. _____ Wrap plates in plastic wrap and tape in notebook.

Notes:
1. Depending on the type of TLC needed, there are three basic types of TLC plates to use. For TLC analysis, use aluminum-backed EM Science F254 plates (EM Science 5554/7). For small preparative TLC, use plastic-backed EM Science F254 plates (EM Science 5735/7). For large preparative TLC, use glass-backed EM Science F254 plates (EM Science 13792/7). For performing preparative TLC, please see SOP SP032 Preparative Thin Layer Chromatography.
2. Fresh HPLC-grade chemicals, preferably Burdick & Jackson, make the best TLC solvents systems. Commons systems are $CHCl_3$-CH_3OH-H_2O (60:30:6) for PIM preparation/analysis and $CHCl_3$:CH_3OH:NH_4OH (80:20:2) for TDM preparation/analysis. Fresh solvents are also important for solubilization of lipids for TLC analysis.
3. For small TLC's (5 x 10 cm or 10 x 10 cm) use a small Kontes tank (Kontes 416180-1020). For large TLC's (20 x 10 cm or 20 x 20 cm) use a large Kontes tank (Kontes 416180-0000).
4. There are numerous detection sprays, based on what molecule is to be detected. See SOP for charring spray, α-napthol and Dittmer-Lester for details. Additionally, F_{254} plates may be viewed under low and high wave ultraviolet light, and all plates may be placed in a tank with iodine crystals, which will temporarily stain some compounds.
5. For basic TLC analysis, cut aluminum-backed plates to 5 x 10 cm, 10 x 10 cm or 20 x 10 cm.
6. It is best to make all spots one centimeter apart, and no closer than one centimeter to plate edges (to prevent the solvent front from migrating unevenly).
7. Ideally, compound should be suspended in $CHCl_3$-CH_3OH (2:1) at a concentration around 5-10 μg per μl, and 10-30 μg should be applied to plate. Each spot should be applied as a small band. New glass capillaries should be used for each compound
8. Wash tank using mild detergent soap. Rinse each side three times with hot water, three times with deionized water, once with HPLC-grade CH_3OH and dry under compressed air. Then, rinse each surface with HPLC-grade acetone and dry under compressed air.
9. Make approximately 10 ml for small TLC tanks and 100 ml for large TLC tanks.
10. Tank should be sealed as best as possible to minimize leakage of solvent fumes. Preferably, place tank in area with low air flow.
11. Place a partially open box in a chemical fume hood and cover with foil. This will provide a place to set the TLC plate while spraying, and protect the inside of the hood from oxidizing chemicals.
12. All TLC plate developers will fade with time; Dittmer-Lester may disappear within minutes, so it is important to document TLC results as soon as possible.

References:
Besra, GS (1998) Preparation of Cell-Wall Fractions from Mycobacteria. Methods in Molecular Biology, Vol 101: Mycobacterial Protocols (Parish T and Stoker, NG ed), Humana Press Inc, Towata NJ, pp 91-107.

Slayden, RA and Barry 3rd, CE (2001). Analysis of the Lipids of Mycobacterium tuberculosis. Mycobacterium tuberculosis Protocols (Parish T and Stoker, NG ed), Humana Press Inc, Towata NJ, pp 229-246.

Belisle, JT, Vissa VD, Sievert T, Takayama K, Brennan PJ, and Besra GS. Role of the Major Antigen of Mycobacterium tuberculosis in Cell Wall Biogenesis. Science (276): pp 1420-1422.

Dittmer, JC and Lester, RL. A Simple, Specific Spray for the Detection of Phospholipids on Thin-Layer Chromatography. Journal of Lipid Research (15): pp 126-127.