

**SOP: PP037.3**  
**Updated 4/17/17**

### **Purification of PDIM**

#### **Materials and Reagents:**

1. Chemical fume hood
2. Magnetic stir plate/bars
3. Glass bottles, 100-500 ml
4. Glass funnel
5. Rotary evaporator
6. Round bottom glass flasks, 250-500 ml
7. TLC plates, preparative (Merck 1.05715.0001)
8. TLC sheets (Merck 1.05548.0001)
9. Kontes TLC tanks, large and small
10. TLC aluminum racks, large and small
11. Chloroform (note 1)
12. Methanol
13. Water
14. Petroleum ether
15. Ethyl acetate
16. Acetone

#### **Protocol:**

- 1.\_\_\_\_\_ Apply Folch-washed total lipid to 10-15 preparative TLC plates using 15-20 mg total lipid per plate (note 2).
- 2.\_\_\_\_\_ Develop plates with 98/2 petroleum ether/ethyl acetate (note 3).
- 3.\_\_\_\_\_ Scrape PDIM-specific bands (top 3) and extract silica 2X with 2:1 chloroform/methanol, 10 mL each.
- 4.\_\_\_\_\_ Evaluate purity of combined, crude PDIM with analytical 2D TLC (note 4).
- 5.\_\_\_\_\_ Obtain dry weight of this first crude extract.
- 6.\_\_\_\_\_ Apply to preparatory TLC plate(s) for final clean-up, using no more than 20 mg per plate.
- 7.\_\_\_\_\_ Develop and extract as before (steps 2-3).
- 8.\_\_\_\_\_ Apply 50 µg per analytical TLC plate for final purity check.
- 9.\_\_\_\_\_ If purity is not good, repeat the clean-up process (steps 6-8).
- 10.\_\_\_\_\_ Submit 25 µg purified PDIM for MALDI-TOF analysis in positive mode (note 5).
- 11.\_\_\_\_\_ Make 0.5 mg aliquots and store dried at -80°C.

#### **Notes:**

1. All organic solvents should be HPLC grade and used in the chemical fume hood.
2. See SOP PP018 for Folch-wash procedure. Apply 50 µg PDIM control on edge of at least one plate to visualize PDIM-specific bands. Work in batches of 4-6 plates. Extraction can proceed the next day while new plates are being developed.
3. Score prep plates 1 cm from top with spatula before running. Run time ~ 1 hour. See SOP SP032 for a more detailed description of running preparative TLC. The edges of the plate should be sprayed with CuSO<sub>4</sub> (also referred to as charring spray, prepared according to SOP R011) and charring for visualization.
4. Cut aluminum-backed TLC sheets down to 10 x 10 cm. Run 1<sup>st</sup> dimension is 98/2 petroleum ether/ethyl acetate (x2), 2<sup>nd</sup> 98/2 petroleum ether/acetone. Should see three distinct spots. Visualize with CuSO<sub>4</sub> (SOP R011) and charring, according to SP033.

5. PDIM should be spotted using DHB matrix. There should be a cluster of peaks from 1306 to 1481 m/z, with peaks separated by 14 amu representing methylene groups.