Glycolipid extraction

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Reagents:
-Chloroform
-Methanol
-HPLC grade Water

Equipment/Apparatuses:
- Dounce homogenizer
- Vortex mixer
- Cold centrifuge
- N_2 evaporator
- Glass threaded culture tube (conical bottom)
- Phenolic cap with PTFE-Faced rubber liner
- Glass pipett

Procedures:

1. Extract glycolipids from sample by adding a mixture of chloroform:methanol:water (4:8:3, v/v/v). Tissue disruption is recommended to improve the recovery of glycolipids.

2. Centrifuge at 2,500 rpm for 10 min at 4°C to precipitate insoluble material (protein).

3. Transfer the supernatant into a new screw top glass tube (13 x 100 mm). This contains a variety of lipids, phospholipids, glycolipids, cholesterols and free fatty acids.

4. Add C/M/W and vortex.

5. Repeat extraction three times and combine the supernatant in the same glass tube.

6. Dry the extracts under N_2 stream at 40 °C.

Note:

- The extraction process can be stopped at any step and both sample and the extracts can be stored at 4 °C for overnight or -20°C for long storage. Sample preparation can be re-started anytime within the next 7 days.

- The precipitated protein can be used for glycoprotein-glycan analysis or protein ID by standard mass spectrometric approaches.