Mouse Brain Glycolipid Extraction

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Materials & Reagents:

- ~200 mg brain tissue
- Purified water
- Distilled methanol
- Distilled chloroform
- Potter-Elvehjem Teflon-glass homogenizer
- Glass screw-capped tubes with Teflon-lined screw caps
- Glass pipettes (including glass Pasteur pipettes)
- Table-top centrifuge
- Sep-Pak tC18 solid phase extraction cartridge (e.g. WAT036810)
- Glass syringe (5 or 10 ml)
- Nitrogen gas/nitrogen drying apparatus

Method:

1. Weigh brain tissue and place in ice-cold homogenizer.
2. Add 4 volumes (4 ml/g wet weight) water
3. Calculate the "total aqueous volume" as the volume of water added plus 80% of the weight of tissue; e.g. 1 g of tissue would result in 4.8 ml total aqueous volume.
4. Homogenize 10 strokes or until the tissue is homogeneously suspended
5. Add 2.67 volumes (based on the total aqueous volume) of ambient temperature (RT) methanol to the homogenizer, mix thoroughly, transfer to a glass screw-capped tube at RT. Cap and vortex vigorously. Allow mixture to come to RT. All subsequent steps are done at RT or as indicated.
6. Add 1.33 volumes of chloroform (based on the total aqueous volume). Cap and mix vigorously.
7. Centrifuge at 1500 RPM in a table-top centrifuge (~ 450 g) at RT for 15 min.
8. Transfer the supernatant to a fresh screw-capped tube with a graduated pipette. Measure this as the "recovered extract volume".
9. Add 0.173 volumes of water (based on the recovered extract volume), cap, vortex vigorously.
10. Centrifuge at 1500 RPM in a table-top centrifuge (~ 450 g) at RT for 15 min.
11. Transfer the upper phase (~80% of the total volume) to a fresh screw-capped tube using a pulled Pasteur pipette.
12. During the above centrifugations, prewash a tC18 Sep-Pak with ~ 3 ml each of the following: (i) chloroform-methanol-water (2:43:55), (ii) methanol:water (1:1), (iii) methanol, (iv) methanol:water (1:1), and (v) chloroform-methanol-water (2:43:55).
13. Load the upper phase from the partition onto the pre-washed Sep-Pak. Wash with 3 ml each of the following: (i) chloroform-methanol-water (2:43:55), (ii) methanol:water (1:1).
14. Elute the gangliosides with 3 ml of methanol and collect into a fresh screw capped tube.

15. Evaporate to dryness under a stream of dry nitrogen at 45 °C
16. Redissolve in 2.5 volumes of methanol, based on the original tissue weight adjusting for recovery of "recovered extract volume".

NOTES:

- Add the methanol, mix, and allow to come to RT prior to adding the chloroform. This maximizes protein precipitation, and therefore final ganglioside purity
- The original additions are calculated to give a chloroform-methanol-aqueous ratio of 4:8:3, which maximize ganglioside recovery.
- After centrifugation to remove precipitated proteins and nucleic acids, water is added to give a final chloroform-methanol-aqueous ratio of 4:8:5.6 ratio, which was discovered to maximize partitioning of gangliosides into the upper phase.
- For brain, the estimated concentration of lipid-bound sialic acid (e.g. sialic acid on gangliosides) is 2.5 µmol/g fresh weight. The concentration of ganglioside sialic acid in the final purified methanol solution, therefore, is estimated to be 1 nmol/µl, an appropriate amount for TLC spotting and detection.
- Tissue ganglioside concentrations vary greatly, with brain gray matter being the highest, spleen (for example) having about 1/10 as much, and fat tissue 1/100 as much per g wet weight.