Removal of free lipids from a GSL mixture

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Reagents:
-n-Hexane

Equipment/Apparatuses:
- Cold centrifuge
- Vortex mixer
- N_2 evaporator
- Ice bucket
- Glass pipette

Procedures:

1. Add 1ml of n-hexane into the dried glycolipid and vortex.
2. Put on ice for 15 min
3. Centrifuge at 2,500 rpm (600 x *g*) for 15 min to precipitate glycolipids at 4 ^{o}C.
4. Pipette off supernatant containing free lipids.
5. Dry precipitate (glycolipid) under N_2 stream at 40^{o}C

Note:

- The dried sample is ready for permethylation and can be stored at -20 °C until use.
- The supernatant (free lipid fraction) may be kept in a new glass tube and analyze the contents (carryover GSLs, etc) as necessary.

- HPTLC analysis is recommended in order to estimate glycolipid amount/composition prior to MS analysis (**Figure 1**). Qualitative glycolipid mixture is useful to evaluate glycolipid component. Matreya glycolipid standard comes with 1mg/mL or 0.5mg/mL solution in C/M/W. Apply 1 - 2ug of the solution onto a HPTLC plate together with interest of glycolipid sample.

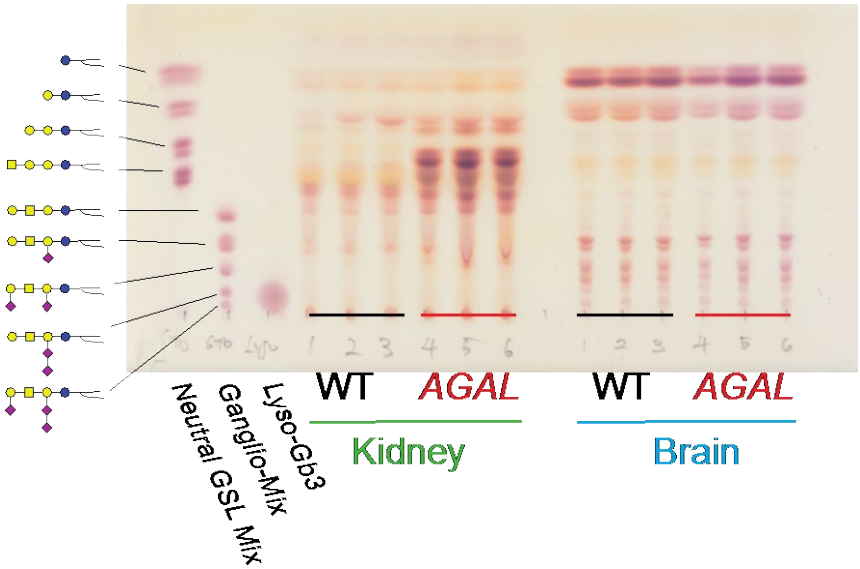

Figure 1. An example of Thin layer chromatography (TLC) analysis result[1]

Reference:

1. Miller, J.J., et al., *alpha-Galactosidase A-deficient rats accumulate glycosphingolipids and develop cardiorenal phenotypes of Fabry disease.* FASEB J, 2018: p. fj201800771R.

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