

## Lipid Extraction for Mass Spec

1. Take 6-8 leaves (or 1-3 whole little plants); quickly immerse in 3.0ml 75°C isopropanol with 0.01%BHT for **15 min**. Use a 50ml (25x150mm) glass tube with a Teflon-lined screw cap.
2. Add 1.5ml chloroform and 0.6ml water, vortex; then agitate (shaking incubator) at room temperature for **1 hour**. Transfer (long, glass Pasteur pipettes) lipid extracts to glass screw-cap (Teflon-lined) tubes.
3. Add 4.0ml chloroform/methanol (2:1) with 0.01%BHT; shake 30min. Repeat this extraction procedure on all samples until the leaves of every sample become white. (Use one pipette in each sample for all extractions, leaving them in the removed extract while extracting the remaining materials.) It's OK to leave for somewhat **longer than 30 min** on later extractions – one of these should go overnight for really thorough extraction. Usually it takes about 5 extractions, including the one with the isopropanol.
4. Add 1.0ml 1 M KCl to the combined extract, vortex or shake, centrifuge to break the phases, discard upper phase.
5. Add 2.0ml water, vortex or shake, centrifuge, discard upper phase.
6. Store at -20°C (freezer). When you are ready to prepare for shipping, evaporate completely, and redissolve in about 1.0 ml chloroform. Transfer to **2.0 ml clear glass vial with Teflon-lined screw cap** (for example: Clear Glass; 2mL; Solid PTFE-lined Cap; 1/2 dr. Fisher Scientific catalog #03-391-7A Thermo Scientific No.:B7800-1). **\*ALWAYS\*** Let us know before you send your samples: [mrroth@ksu.edu](mailto:mrroth@ksu.edu)

Evaporate completely again, cap snugly, and pack in small box or bubble wrap for protection from breaking.

7. Dry the extracted leaves at 105°C oven overnight, weigh to 6 decimal places (0.000000g) for dry weight. Enter these weights on the KLRC sample data form. Place a completed form with the samples for accurate identification upon arrival. Return a completed form to my email for your folder. Samples will not be analyzed without a completed KLRC form.

8. Ship the samples on dry ice, with overnight delivery, to my attention:

Mary Roth  
Kansas Lipidomics Research Center  
Division of Biology  
510 Ackert Hall  
Kansas State University  
Manhattan, KS 66506-4901 USA  
(785) 532-5756

9. Samples from outside the USA need to have a Customs Release Letter enclosed with the shipping papers. It makes the delivery much faster.

If dry ice is not possible or too costly, samples have been received at ambient temperature from overseas and seem to be fine.

**It takes at least 6 to 8 weeks before the data is ready to send.**