Lipid Extraction Method for Arabidopsis (and other) Leaves (Method 1)

This procedure is generalized. Use of smaller samples is possible. This procedure can be adapted for other plant tissues, including roots, stems, flowers, and siliques. An example of this method as applied to leaves of a monocot is available here. Please contact Mary Roth at mrroth@ksu.edu (please include a subject line) to inquire.

1. Take 1 to 8 leaves (or up to 3 whole little plants- see NOTE #2); quickly immerse in 3 ml 75ºC (preheated) isopropanol with 0.01% BHT (butylated hydroxytoluene, e.g., Sigma B1378) and continue to heat for **15 min**. Use a 50 ml (25 x 150 mm) glass tube with a Teflon-lined screw cap. NOTE #1: It is extremely important that the plants be extracted immediately after sampling and that the isopropanol be preheated. Plants have very active phospholipase D, which is activated upon wounding; failure to place the sampled tissue quickly into hot isopropanol will result in generation of phosphatidic acid. NOTE #2: To do the analysis, only a fraction of an Arabidopsis leaf is needed. Use of more tissue reduces variability among samples; use of smaller amounts of tissue can provide information about individual plants and plant tissues. Dry weights of 5 to 30 mg, measured as in step 6, are recommended.
2. Add 1.5 ml chloroform and 0.6 ml water, vortex; then agitate (shaking incubator) at room temperature for **1 hour**. Transfer (long, glass Pasteur pipettes) lipid extracts to glass tubes with Teflon-lined screw-caps.
3. Add 4 ml chloroform/methanol (2:1) with 0.01% BHT; shake 30 min. Repeat this extraction procedure on all samples until the leaves of every sample become white, but be sure to extract each sample the same number of times. (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 the tubes shaking for somewhat longer than 30 min on later extractions, and leaving one of the extractions shaking overnight is a good idea for difficult-to-extract tissues. Usually you will need about 5 extractions, including the one with the isopropanol.
4. Optional back-washes: Add 1 ml 1 M KCl to the combined extract, vortex or shake, centrifuge, discard upper phase. Add 2 ml water, vortex or shake, centrifuge, discard upper phase. These backwashes will yield a cleaner lipid sample, but small amounts of the more polar lipids, such as lysolipids, will be lost.
5. Fill tubes with nitrogen, store at -20°C (freezer). When you are ready to prepare for shipping, evaporate completely under nitrogen gas or a speedvac, 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; 2 mL; solid PTFE-lined cap; 1/2 dr., Fisher Scientific catalog #03-391-7A, Thermo Scientific No.: B7800-1). Evaporate solvent from the samples in the 2-ml vials before shipping. **ALWAYS** let us know before you send your samples: mrroth@ksu.edu
6. After contacting me and sending a completed KLRC-sample-data form, ship dried overnight, on dry ice, to my attention:

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7. Dry extracted leaves at 105°C oven overnight; weigh for "dry" weights preferably using a balance that weighs (in grams) to 6 decimal places (i.e., micrograms).