

Extraction of Arabidopsis Seeds using a combination of Leaf and Animal Protocols

NOTES: Use glass tubes with Teflon-lined screw caps. Use a Dounce-type homogenizer – ground glass to ground glass. Use glass pasteur pipets for transferring seeds/solvent. Volumes may be doubled for larger samples or ease of homogenizing.

- 1) Weigh seeds. Lyophilizing first may be best for consistent dry weight.
- 2) Drop seeds into 1.0 ml 75°C isopropanol with 0.01%BHT. Heat 15 minutes. Let cool. (Hot isopropanol inactivates phospholipase enzymes.)
- 3) Transfer to homogenizer; homogenize thoroughly. Transfer to 17 ml glass tube for extraction. Rinse homogenizer with 1.0 ml chloroform and 1.0 ml methanol to recover all the seed parts/lipids; add to isopropanol/seed mix in tube. Add 0.8 ml water to solvent/seed mix. Shake well. There should be one phase.
- 4) Add 1.0 ml chloroform and 1.0 ml water. Shake well. Centrifuge to split phases.
- 5) Remove lower layer (chloroform and lipids). SAVE to clean glass tube.
- 6) Add 1.0 ml chloroform, shake, and centrifuge. SAVE this lower layer in same tube as first.
- 7) Add 1.0 ml chloroform, shake, and centrifuge. SAVE this lower layer in same tube as above.
- 8) Add small amount (0.5 ml) 1 M KCl to chloroform/lipids. Shake well; centrifuge. Remove thin water layer on TOP and discard.
- 9) Add small amount (1.0 ml) water to chloroform/lipids. Shake well; centrifuge. Remove thin water layer on TOP and discard.
(This backwash removes any proteins and carbohydrates that may have been carried through the extraction.)
- 10) Dry to small volume. Transfer to 2.0 ml 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), dry completely, fill vial with nitrogen gas, and ship overnight on dry ice.
- 11) Enter the 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.

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

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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 are ready to send.