Homogenization Protocols

What a good homogenate looks like

(Daphnia may not form the pellet since the body seems to readily disentegrate)

**Tip: When using the same homogenates to run multiple assays, aliquot appropriate volumes into several tubes to use separately. For example, if planning to do energy stores and all the G-6-P branch point enzyme assays, set up eight tubes, the first for PRO/TRI/GLY with 250 μL and the remaining seven with 95 μL for the enzyme assays. These numbers are approximate – they are NOT hard and fast rules.*

Finally, below are some helpful tips for performing these assays in general:

- All homogenates should be kept on ice whenever possible. Homogenates taken from the -80 ^{o}C freezer should be left in a microfuge rack at room temperature just long enough for them to melt and then placed on ice.
- It is a good idea to plan the plate layout on paper before actually loading a plate. For the purposes of data analysis, it is easiest to load a plate column by column. For example, fill up column 1 by loading A1, then B1, then C1, to the end of the column, and then go to column 2, A2, B2, C2, etc.,
- When loading samples into the plate, make sure that each homogenate is mixed immediately before loading. For all enzyme kinetic assays, do not vortex because it will denature the enzymes. Instead, mix by gently pipetting up and down before. Homogenates used in energy stores assays (ATP, PRO, TRI, GLY) can be vortexed. (The BCA protein assay method we use does not depend on the enzyme's tertiary structure.)
- If doing all three potassium phosphate energy stores assays (PRO, TRI, GLY) on a given set of homogenate, plan to do all three assays on the same day. (Keep the homogenates on ice.) It usually works best to plate TRI first, then PRO, and then GLY.
- Change tips between loading every sample.

Good luck!

❍ For ATP Assay

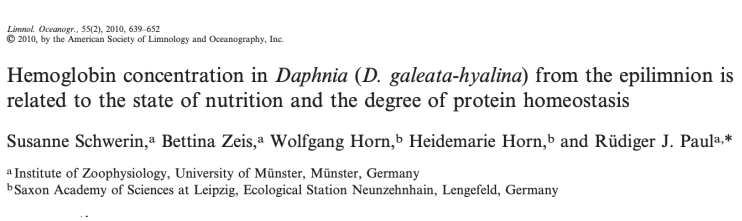
Buffer: 6M Guanidine-HCl

(high molarity guanidine keeps the ATP stable by degrading ATPases)

- (Turn on the heating block on the high setting, and heat to 95 °C)
- Collect and weigh 10 flies of the same gender
- Place flies into a labeled microfuge tube on ice
- Add 100 μL buffer
- Homogenize for 20 seconds using overhead rotor (speed ~3) until there are no big fly particles (the cuticles never grind completely)
- Add 100 μL more buffer
- Heat the tubes in the heating block at 95 °C for 5 minutes
- Centrifuge at 14,000 rpm for 5 minutes (4 °C)
- Back on ice, pipet out the supernatant into a fresh microfuge tube
- Discard tube with the pellet
- Store supernatant at -80 °C. Do not refreeze.

**Tip: Aliquot the supernatant into two separate tubes to use as a back-up or for a duplicate assay.*

** Reference for protocol specific to *Daphnia*.



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Hemoglobin concentration in *Daphnia* (*D. galeata-hyalina*) from the epilimnion is related to the state of nutrition and the degree of protein homeostasis

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A comparative analysis of the fatty acid composition of sexual and asexual eggs of *Daphnia magna* and its plasticity as a function of food quality

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❍ For all other assays (Trilycerides, Protein, Glycogen):

Buffer: 0.01M KH_2PO_4, 1 mM EDTA (pH 7.4) (in fridge – labeled with my name and 'Protein Assay')

- Label microcentrefuge tubes on the lid AND on the side with the number of your sample
- Collect and weigh 10 flies (10 *Daphnia*) of the same age/gender
- Place flies into a labeled microfuge tube on ice
- Add 300 μL buffer (for smaller ***Daphnia*, try 200 uL** instead)
- Homogenize for ~ **60 seconds** using overhead rotor (speed ~3) until there are no big fly (*Daphnia*) particles (the cuticles never grind completely)
- Add 700 μL more buffer (for smaller ***Daphnia*, try 500 uL** instead)
- Centrifuge at 2000 rpm for **20 minutes** (4 °C)**
- Back on ice, pipet out the supernatant into a fresh microfuge tube
- Discard tube with the pellet (for *Daphnia*, you may NOT have a pellet)
- Distribute the homenate into three separate vials for metabolic assays: (see graphical illustration on the next page)
    - **250 for triglycerides**
    - **200 for glycogen**
    - **200 for protein**
        - Label vials (# and assay on side and on the top give the # and a descriptive abbreviation, e.g., P for Protein) to match the sample number and assay
          e.g., # 1 Tri, # 1 Glycogen, # 1 Protein

- Samples are now ready to run the metabolic assys. If not running assays on the same day, store supernatants at -80 °C (box labeled Lauren, Rachel, Jess) until ready to run the metabolic assays **Do not thaw/refreeze**.

Step A

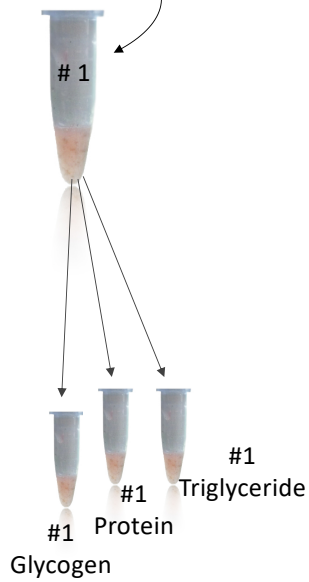
Step B

(A) Dry & Weigh enough *Daphnia* to reach a combined weight of **0.65 mg (usually 10 *D. dentifera*)**

** Be consistent in the length of time You dry each *Daphnia* otherwise, the weights will be super different and useless.

(B) Then combine these (10) *Daphnia* into a single centrifuge tube (this will allow us to have enough protein that we can actually measure it).

(C) Once you have added the buffer and spun down Homogenate. Aliquot the concentrated solution into Separate centrifuge tube vials for each assay: Glycogen, triglyceride, & protein



Data sheet: Homogenate prep.
Animals are from which spore farm: _____

Date	Weight	# Animals	Treatment	Initials