Protocol for *D. melanogaster* Larvae Homogenization in the Bullet Blender™

The protocol described in this document is for the use of the Bullet Blender™ for the homogenization of Drosophila melanogaster larvae. This protocol does not specify a particular buffer - you may choose which is most appropriate for your downstream application (nucleic acid isolation, protein extraction, etc.).

Materials Required: Drosophila larvae, Bullet Blender™, homogenization buffer,

pipettor, microcentrifuge tubes, and <u>0.5mm glass beads (part</u>

number GB05).

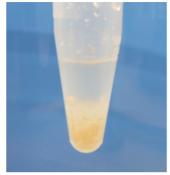
Instructions

- 1. If you have not already, wash Drosophila larvae 3x with 1ml PBS or other buffer, as appropriate, to remove food, surface bacteria, and other contaminants.
- **2.** Aspirate the larvae, or remove as much liquid as possible with a pipette.
- **3.** Place 10-300mg of larvae into microcentrifuge tubes.
- **4.** Add an equal mass of 0.5mm glass beads to the tube. One scoop \approx 77mg.
- 5. Add 2 volumes of buffer for every mass of larvae (for example, with 100mg of larvae, use 200μl of buffer).
- **6.** Close the microcentrifuge tubes.
- 7. Place tubes into the Bullet Blender™.
- **8.** Set controls for **SPEED 8** and **TIME 3** minutes.
- **9.** Remove tubes from the instrument.
- 10. Visually inspect samples, if homogenization is unsatisfactory, run for another two minutes at the SPEED 10.
- 11. Proceed with your downstream application.

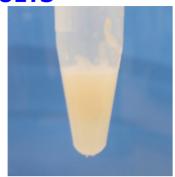
SAFETY NOTE!!!

When using a centrifuge to separate your homogenate from the debris and beads, make sure your tubes are balanced.

TYPICAL RESULTS



before



after



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Ponceau-stained protein gels. 35mg of larval Drosophila epidermal tissue was homogenized with a pestle (a) or a Bullet Blender Blue (b). Tissue was pelleted, 30 mL of 2x Laemmli buffer was added, and 1/3 scoop of 0.5 mm zirconium silicate beads was added. Samples processed in the Bullet Blender were homogenized for 2 minutes at speed 8. Protein extraction is increased dramatically when the samples are homogenized using a Bullet Blender. Data courtesy of Laura Stevens at RPI.



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