# 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 0.5mm glass beads (part 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 a volume of beads equal to the mass of tissue. **NOTE:** 100mg $\cong$ 100μL.
5. Add 2 volumes of buffer for every mass of larvae.
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.**
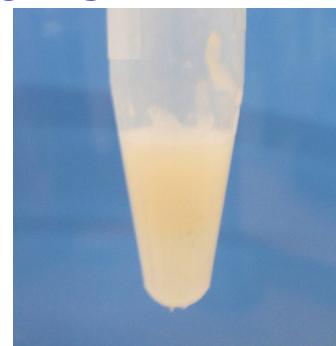11. Proceed with your downstream application.

## SAFETY NOTE!!!

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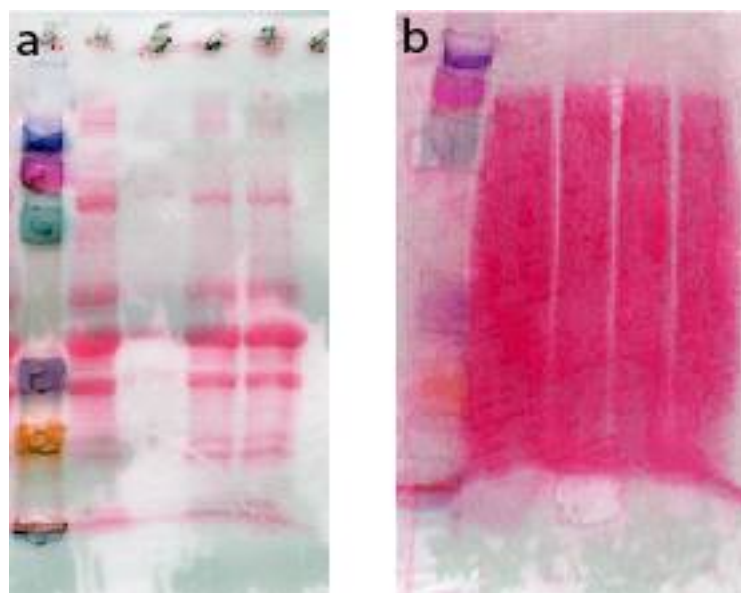
## TYPICAL RESULTS



**before**



**after**

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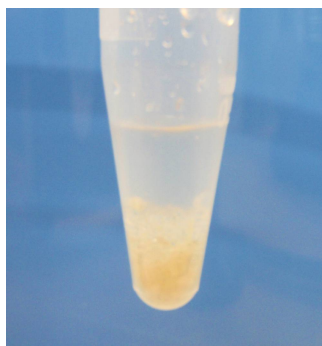
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Ponceau-stained protein gels. 35mg of larval *Drosophila* epidermal tissue was homogenized with a pestle (a) or a Bullet Blender Blue (b). Tissue was pelleted, 30 mL of 2x Laemmli buffer was added, and 1/3 scoop of 0.5 mm zirconium silicate beads was 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.