

Homogenization in the Bullet Blender® 5 Protocol for *D. melanogaster* Larvae

The protocol described in this document is for the use of the Bullet Blender® 5 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® 5, homogenization buffer, pipettor, 5mL Axygen® brand 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 100-1000mg of larvae into 5ml tubes.
4. Add a volume of beads equal to the mass of tissue. **NOTE:** 100mg \approx 100 μ L.
5. Add 0.2mL to 2.0mL buffer (2 volumes of buffer for every volume of sample).
6. *Tightly* screw the centrifuge tubes closed and place them into the Bullet Blender®.
7. Set controls for **SPEED 7** and **TIME 2** minutes. Press start.
8. After the run, remove the tubes from the instrument.
9. Visually inspect samples, if homogenization is unsatisfactory, run for another two minutes at **SPEED 8**.
10. 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.