NEXTADVANCE

Protocol for Zebrafish (*D. Rerio*) Homogenization in the Bullet Blender®

The protocol described in this document is for the use of the Bullet Blender® for the homogenization of embryonic or larval zebrafish (*Danio rerio*). This protocol has been tested with fish younger than one week post-fertilization, but should be appropriate for fish up to about one month post-fertilization. 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: zebrafish, Bullet Blender®, homogenization buffer, pipettor, microcentrifuge tubes, and Zirconium Oxide beads (0.5mm or 1.0mm).

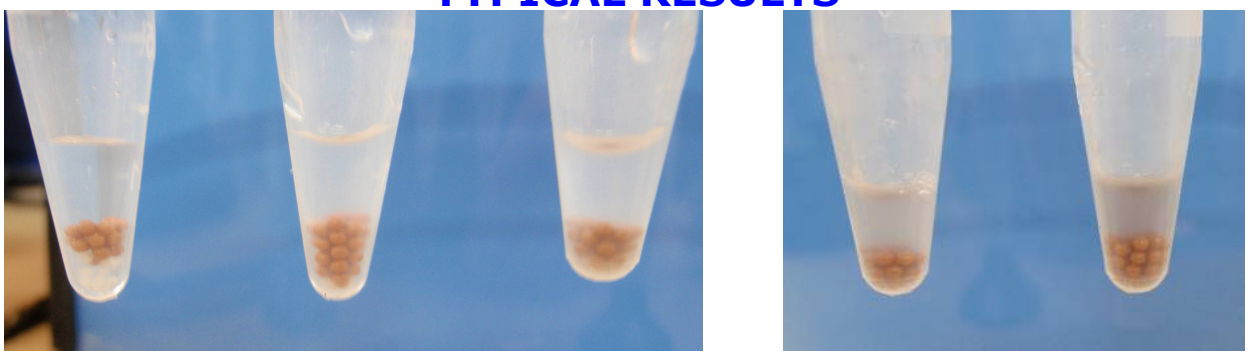
Instructions

1. Place 10-300mg of zebrafish into microcentrifuge tubes. **NOTE:** Unhatched fish do not require prior dechorionation.
2. Add a mass of zirconium oxide beads (0.5mm or 1.0mm) to the tube equal to 2x your mass of sample (so for 50mg of zebrafish, add 100 mg of beads). One scoop of beads ≈ 180mg.
3. Add 2 volumes of buffer for every mass of fish (for example, with 100mg of fish use 200µl of buffer).
4. Close the microcentrifuge tubes.
5. Place tubes into the Bullet Blender®.
6. Set controls for **SPEED 8** and **TIME 3** minutes.
7. Remove tubes from the instrument.
8. Visually inspect samples, if homogenization is unsatisfactory, run for another two minutes at the **SPEED 8.**
9. 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 (2dpf) **after (2dpf)** **after (2dpf)** **after (4dpf)** **after (6dpf)**

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