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Protocol for Brain Tissue Homogenization in the Bullet Blender™

The protocol described in this document is for the use of the Bullet Blender™ for the homogenization of brain tissue (from a variety of animals). Note that the time and speed settings may differ due to the variation in consistency/texture of tissue from species to species. 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: brain tissue, Bullet Blender™, homogenization buffer, pipettor, microcentrifuge tubes, and 0.5 mm glass beads (part number GB05).

Instructions

1. Cut brain tissue into appropriately sized pieces for analysis (50mg-300mg) and place into a microcentrifuge tube. Typical sample size: 100mg.
2. **OPTIONAL:** Wash tissue 3x with ~1mL PBS. Aspirate. **NOTE:** This step removes external contaminants (blood, etc.).
3. Add glass beads (0.5mm) to the tube. Use a mass of beads equal to your mass of tissue. **NOTE:** 100mg ≅ 50µL of 0.5mm glass beads. It is acceptable to use denser beads, such as zirconium silicate or zirconium oxide beads, or beads one size larger, i.e. 1.0 mm beads.
4. Add 0.1mL to 0.6mL buffer (2 volumes of buffer for every mass of tissue).
5. Close the microcentrifuge tubes.
6. Place tubes into the Bullet Blender™.
7. Set controls for **SPEED 6** and **TIME 3** 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 the **SPEED 6.**
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!

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