Bullet Blender® 5
Homogenization Protocol for Brain Tissue

The protocol described in this document is for the use of the Bullet Blender® 5 for the homogenization of brain tissue (from a variety of animals). If you have difficulty with this protocol, cutting your tissue into smaller pieces will help. 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® 5, homogenization buffer, pipettor, 5mL Axygen® brand tubes, and 2.0 mm zirconium oxide beads (part number ZrOB20).

Instructions

1. Cut brain into appropriately sized pieces for analysis (0.1g – 1g).
2. **OPTIONAL:** If desired, wash the tissue 3x with 5mL PBS to remove blood and other contaminants from the tissue.
3. Place sample in 5ML tube and add beads to the tube. Use a volume of beads equal to the mass of tissue. **NOTE:** 100mg ≅ 100µL.
4. Add 0.2mL to 2.0mL buffer (2 volumes of buffer for every volume of sample).
5. *Tightly* screw the centrifuge tubes closed and place them into the Bullet Blender®.
6. Set controls for **SPEED 8** and **TIME 3** minutes. Press start.
7. After the run, remove the tubes from the instrument.
8. Visually inspect samples, if homogenization is unsatisfactory, run for another two minutes at **SPEED 9**
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.