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Protocol for Lymphatic Tissue Homogenization in the Bullet Blender™

The protocol described in this document is for the use of the Bullet Blender™ for the homogenization of lymph nodes (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: lymph node tissue, microcentrifuge tubes, Bullet Blender™, homogenization buffer, pipettor, and 0.5mm zirconium oxide beads (part number ZrOB05)

Instructions

1. Cut lymph tissue into appropriately sized pieces for analysis (50mg-300mg) and place into a microcentrifuge tube. Lymph nodes vary widely in size (from 5mg to 150mg).
2. **OPTIONAL:** Wash tissue with ~1mL PBS. Aspirate. **NOTE:** This step removes external contaminants (blood, etc.).
3. Add zirconium oxide beads (0.5mm). Use a mass of beads equal to your mass of tissue. **NOTE:** For either bead type, **NOTE:** 100mg of beads ≅ 50µL
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 8** and **TIME 3** minutes. Press **Start**.
8. After the run, remove tubes from the instrument.
9. Visually inspect samples. If homogenization is unsatisfactory, run for another two minutes at the **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.

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