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, Bullet Blender®, microcentrifuge tubes, Navy bead lysis kit/Green bead lysis kit/stainless steel beads (1.6mm, product number SSB16 or 0.9-2.0mm blend, product number SSB14B), homogenization buffer, and pipettor.

Instructions

1. Cut lymph tissue into appropriately sized pieces for analysis (10mg-300mg).
2. OPTIONAL: Wash tissue with ~1mL PBS. Aspirate. NOTE: This step removes external contaminants (blood, etc.).
3. a. *Samples 50mg or greater*
   Place the sample in Navy bead lysis kit tube.
   b. *Samples less than 50mg*
   Place the sample in Green bead lysis kit tube.
   c. *Alternate protocol step for bulk beads*
   Place sample in microcentrifuge tube and add beads to the tube. Use a volume of beads equal to the mass of tissue. NOTE: 100mg ≅ 100µL.
4. Add 0.025mL 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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