

Protocol for Splenic (Spleen) Tissue Homogenization in the Bullet Blender™

The protocol described in this document is for the use of the Bullet Blender™ for the homogenization of splenic (spleen) 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: splenic tissue, Bullet Blender™, homogenization buffer, pipettor, microcentrifuge tubes, and [0.5mm zirconium oxide beads \(part number ZrOB05\)](#).

Instructions

1. Cut spleen tissue into appropriately sized pieces for analysis (20mg-300mg) and place into a microcentrifuge tube. One BALB/c mouse spleen \cong 100mg.¹
2. **OPTIONAL:** Wash tissue 3x with \sim 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 2x mass of tissue. **NOTE:** 100mg of beads \cong 35 μ L.
4. Add 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 10**.
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.

1) <http://www.jove.com/pubmedgen/default.aspx?PDF=&ID=1029>