

# Protocol for Cartilaginous Tissue Homogenization in the Bullet Blender®

The protocol described in this document is for the use of the Bullet Blender® for the homogenization of cartilage (from a variety of animals). Note that the time and speed settings, and digestion parameters may differ due to the variation in consistency/texture of tissue from species to species. **Particularly tough cartilage samples may require enzymatic pretreatment with collagenase and / or hyaluronidase in order to achieve good homogenization.** This protocol does not specify a particular homogenization buffer - you may choose which is most appropriate for your downstream application (nucleic acid isolation, protein extraction, etc.).

**Materials** cartilage, Bullet Blender<sup>®</sup>issue, Bullet Blender<sup>®</sup>, homogenization buffer, pipettor, microcentrifuge tubes, and Navy bead lysis kit/0.9-2.0mm stainless steel bead blend (product number SSB14B)

## Instructions

1. Dice cartilage tissue into small pieces. **Note:** Pieces larger than 30mg may require enzymatic digestion (see optional steps 3 and 4).
2. **OPTIONAL:** Add 1mL hyaluronidase (H-3506, Sigma Chemical, St. Louis, MO) to sample and incubate (15 minutes at 37°C, on Next Rocker). Wash the sample with 1mL PBS. Centrifuge at 1000g for 5 minutes.
3. **OPTIONAL:** Add 1mL collagenase, type II (CLS2, Worthington, Lakewood, NJ) to sample and incubate (2 to 4 hours at 37°C, on Next Rocker). Wash the sample with 1mL PBS. Centrifuge at 1000g for 5 minutes. Aspirate supernatant.
4. a. *Protocol step using pre-loaded tubes*  
Place the sample in Navy bead lysis kit tube.  
b. *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  $\cong$  100 $\mu$ L.
5. Add 0.1mL to 0.6mL homogenization buffer (2 volumes of buffer for every mass of tissue).
6. Close the microcentrifuge tubes.
7. Place tubes into the Bullet Blender<sup>®</sup>.
8. Set controls for **SPEED 6** and **TIME 5** minutes. Press **Start**.
9. After the run, remove tubes from the instrument.
10. Visually inspect samples. If homogenization is unsatisfactory, run for another five minutes at the **SPEED 6**.
11. 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.**

This protocol is a modified version of the publication "Cartilage Tissue Engineering for Laryngotracheal Reconstruction: Comparison of Chondrocytes from Three Anatomic Locations in the Rabbit" *Tissue Eng.* 2007 April ; 13(4): 843-853.