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®, homogenization buffer, PBS, pipettor, microcentrifuge tubes, and Navy bead lysis kit/0.9-2.0mm stainless steel bead blend (product number SSB14B) and 3.2mm stainless steel balls

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 volume of tissue. OPTIONAL: Add 1-5 3.2mm stainless steel balls to the tube.
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®.
8. Set controls for SPEED 9 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 10.
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.

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