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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. 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 Required: cartilage, aspirator, Bullet Blender™, homogenization buffer, pipettor, testicular hyaluronidase (H-3506, Sigma Chemical, St. Louis, MO), trypsin-EDTA (0.25%, Invitrogen, Carlsbad, CA), collagenase type II (CLS2, Worthington, Lakewood, NJ), Next Rocker platform rocker, microcentrifuge tubes, and 0.5mm zirconium oxide beads (part number ZrOB05)

Instructions

1. Dice cartilage tissue (20-100mg) into small pieces (~2mm squares) and place into a microcentrifuge tube.
2. **OPTIONAL:** Wash tissue 3x with ~1mL PBS. Aspirate. **NOTE:** This step removes external contaminants (blood, connective tissue, etc.).
3. Add 1mL hyaluronidase to sample and incubate (15 minutes at 37°C, on Next Rocker). Centrifuge at 1000g for 5 minutes. Aspirate supernatant[1]
4. Add 1mL trypsin-EDTA to sample and incubate (30 minutes at 37°C, on Next Rocker). Centrifuge at 1000g for 5 minutes. Aspirate supernatant.[1]
5. Add 1mL collagenase, type II (2 to 4 hours at 37°C, on Next Rocker). Centrifuge at 1000g for 5 minutes. Aspirate supernatant.[1]
6. Add a mass of zirconium oxide beads (0.5mm) equal to your mass of tissue. **NOTE:** 100mg ≅ 50µL beads.
7. Add 0.1mL to 0.6mL homogenization buffer (2 volumes of buffer for every mass of tissue).
8. Close the microcentrifuge tubes.
9. Place tubes into the Bullet Blender™.
10. Set controls for **SPEED 6** and **TIME 5** minutes. Press **Start**.
11. After the run, remove tubes from the instrument.
12. Visually inspect samples. If homogenization is unsatisfactory, run for another five minutes at the **SPEED 6.**
13. 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.

1 **OPTIONAL:** You may wash the sample with 1mL PBS after the digestion. Centrifuge at 1000*g* for 5 minutes. Aspirate supernate. Continue with protocol.

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