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Protocol for Blood Vessel Tissue Homogenization in the Bullet Blender®

The protocol described in this document is for the use of the Bullet Blender® for the homogenization of blood vessel. The homogenization time and speed settings may differ due to variations 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: blood vessel tissue, Bullet Blender®,homogenization buffer, microcentrifuge tubes, pipettor, and Navy bead lysis kit/Green bead lysis kit/0.9-2.0mm stainless steel bead blend (product number SSB14B)

Instructions

1. Cut tissue into appropriately sized pieces for analysis (10mg-300mg).
2. **OPTIONAL:** Wash tissue 3x with ~1mL PBS. **NOTE:** This step removes external contaminants.
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 0.025 mL to 0.6mL buffer (2 volumes of buffer for every volume of sample).
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.

Reference:

Hou CJ, Tsai CH, Su CH, Wu YJ, Chen SJ, Chiu JJ, Shiao MS, Yeh HI. **Diabetes reduces aortic endothelial gap junctions in ApoE-deficient mice: simvastatin exacerbates the reduction.** J Histochem Cytochem. 2008 Aug;56(8):745-52

This protocol is an adaptation of the protocol used in the above article.

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