Date 05/18/2009

Protocol for Cardiac (Heart) Tissue Homogenization in the Bullet Blender™

The protocol described in this document is for the use of the Bullet Blender™ for the homogenization of cardiac tissue / myocardium (from a variety of animals). Note that the time and speed settings may differ due to the variation in consistency / texture of cardiac 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: heart tissue, saline, aspirator, Bullet Blender™, microcentrifuge tubes, stainless steel beads (1.6mm, product number SSB16 or 0.9-2.0mm blend, product number SSB14B), homogenization buffer, and pipettor.

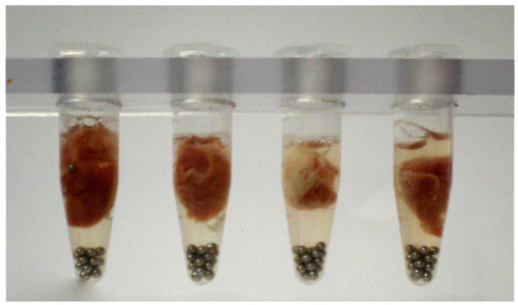
Instructions

1. Cut heart into appropriately sized pieces for analysis (5mg-300mg) and place into a microcentrifuge tube. **NOTE:** Valves or blood vessels will require more vigorous homogenization due to their fibrous nature.
2. **OPTIONAL:** Wash tissue 3x with ~1mL PBS. Aspirate. **NOTE:** This step removes external contaminants (blood, etc.).
3. Add stainless steel beads (1.6mm **OR** 0.9mm-2.0mm blend) to the tube. Use a mass of bead equal to the mass of tissue. **NOTE**: Tissues samples smaller than 30mg require only three (3) 1.6mm stainless steel beads. Do not use the bead blend on these small samples.
4. Add 0.025mL to 0.6mL buffer (2 volumes of buffer for every volume of cells, minimum of 25µL).
5. Close the microcentrifuge tubes.
6. Place tubes into the Bullet Blender™.
7. Set controls for **SPEED 8** and **TIME 4** 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 **SPEED 10.**
10. Proceed with your downstream application.

SAFETY NOTE!!!

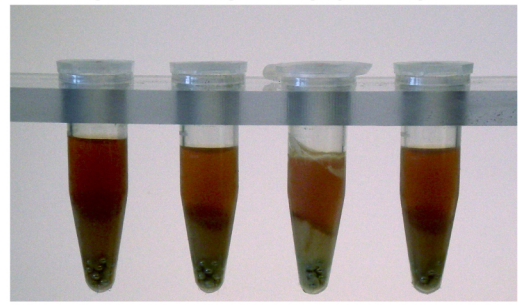
When using a centrifuge to separate your homogenate from the debris and beads, make sure your tubes are balanced!

Heart tissue (0.3g) before homogenization
with stainless beads (1.6mm, 0.3g)
with 0.6mL buffer (0.5% NP-40)



After homogenization,
centrifuged 15minutes @ 14K rpm

protein concentration (mg/mL)
28.1 22.5 28.6 28.4



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