Protocol for Drosophila S2 Cell Culture Homogenization in the Bullet Blender™

The protocol described in this document is for the use of the Bullet BlenderTM for the homogenization of *Drosophila* S2 cell cultures. 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:

Drosophila S2 cells, aspirator, Bullet Blender™, homogenization buffer, pipettor, microcentrifuge tubes, and <u>0.15mm zirconium</u> oxide beads (part number ZrOB015) or 0.1mm glass beads (part number GB01).

Instructions

- **1.** Detach cells from culture dish or flask by your chosen method (trypsinization, scraping, spontaneous detachment, etc.)
- 2. Wash cells from dish with PBS into centrifuge tube
- **3.** Centrifuge cell suspension to yield a cell pellet (1000x g for two minutes).
- 4. Pipette off the supernatant liquid.
- **5.** Inspect the volume of the pellet. It should be $300\mu L$ or less in order to get efficient homogenization.
- **6.** Add a volume of zirconium oxide beads (0.15mm) **OR** glass beads (0.1mm) to the tube equal to about ½ the volume of the pellet.
- 7. Add 2 volumes of buffer for every volume of cells pellet, minimum 25µl.
- 8. Close the microcentrifuge tubes.
- **9.** Place tubes into the Bullet Blender™.
- 10. Set controls for SPEED 8 and TIME 2 minutes. Press Start.
- **11.** After the run, remove tubes from the instrument.
- **12.** Visually inspect samples. If homogenization is unsatisfactory, run for another minute at **SPEED 8.**
- **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.

Results: Western blot with GAPDH stain from 72h culture of Drosophila S2 cells. Cells were homogenized per the above protocol, then the lysate was diluted 10x prior to loading in a 10% tris-glycine gel.

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Scientific Instrument Services, Inc.[™] 1027 Old York Rd. Ringoes, NJ 08551-1039

Phone: (908)788-5550 www.sisweb.com Fax: (908) 806-6631