NEXTADVANCE

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

The protocol described in this document is for the use of the Bullet Blender® 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.).

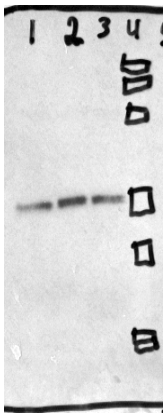
Materials Required: *Drosophila* S2 cells, aspirator, Bullet Blender®, homogenization buffer, pipettor, microcentrifuge tubes, and 0.15mm zirconium oxide beads (part number ZrOB015) or 0.1mm glass beads (part number GB01).

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 300µL 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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