

Protocol for Mammalian 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 mammalian 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: mammalian 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 (200-500g for five minutes at 0°C).
4. Completely aspirate the supernatant liquid. Place tube on ice.
5. Inspect the volume of the pellet. It should be 300µL or less in order to get efficient homogenization.
6. **OPTIONAL (see note below):** Add a volume of zirconium oxide beads (0.15mm) **OR** glass beads (0.1mm) to the tube equal to the volume of the pellet.
7. Add 0.1mL to 0.6mL buffer (2 volumes of buffer for every volume of cells).
8. Close the microcentrifuge tubes.
9. Place tubes into the Bullet Blender®.
10. Set controls for **SPEED 7** and **TIME 2 to 3** minutes. Press **Start**.
11. After the run, remove tubes from the instrument.
12. Visually inspect samples. If homogenization is unsatisfactory, run for another two minutes at the **SPEED 9**.
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.

NOTES

Some cells may be sufficiently lysed in strong buffers (i.e. high salt, high detergent, etc.) without the use of beads. Other cells may require beads regardless of buffer due to their intrinsically stronger membranes. So the **optional** step (number 6) of adding beads is one which we recommend that you empirically determine yourself based on your application and workflow.