



Protocol for *E. coli* Cultures Homogenization in the Bullet Blender™ 50

The protocol described in this document is for the use of the Bullet Blender™ for the homogenization of *Escherichia coli* (or other bacterial) 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: *E. coli*, aspirator, Bullet Blender™ 50, [beads \(zirconium oxide 0.15mm diameter – part number ZrOB015 OR glass 0.1mm diameter – part number GB01\)](#), homogenization buffer, 50mL centrifuge tubes, and pipettor.

Instructions

1. Pour overnight bacterial culture into a 50mL centrifuge tube.
2. Centrifuge culture to yield a cell pellet (2000g for one minute).
3. Completely aspirate the supernatant liquid. Place tube on ice.
4. You may use a larger cell pellet than yielded from 50mL culture by loading more culture, and then repeating steps 1 through 3. **NOTE:** Increasing pellet may require a longer homogenization time to yield the same homogenization efficiency.
5. Inspect the volume of the pellet. It should be 5mL or less in order to get efficient homogenization.
6. Add an equal volume of zirconium oxide beads (0.15mm) **OR** glass beads (0.1mm) to the tube. See **NOTES** below.
7. Add buffer (2 volumes of buffer for every volume of cells).
8. Close centrifuge tubes.
9. Place tubes into the Bullet Blender™.
10. Set controls for **SPEED 8** and **TIME** to **6** 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 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.

NOTES

Different species of bacteria and different applications will be amenable to different bead types. Cell density, cell size, and buffer composition will affect homogenization and variation of the bead selection is an easy way to empirically determine what works best.