Protein extraction (BugBuster)

Materials:

- Liquid culture of cells expressing protein of interest
- BugBuster reagent (5 ml per gram of wet cell paste)

Protocol:

1. Harvest cells from liquid culture by centrifugation at 10,000 × g for 10 min using a weighed centrifuge tube. For small scale extractions (1.5 ml or less), centrifugation can be performed in a 1.5-ml tube at 14,000–16,000 × g. Decant and allow the pellet to drain, removing as much liquid as possible. Determine the wet weight of the pellet.
2. Resuspend the cell pellet in room temperature BugBuster reagent by pipetting or gentle vortexing, using 5 ml reagent per gram of wet cell paste. This typically corresponds to about 2.5 ml per 50-ml culture. For small cultures, use up to 1/5 culture volume for resuspension (e.g., use 300 µl BugBuster for 1.5-ml cultures). There are no adverse effects to using larger volumes of BugBuster, if needed.
3. Incubate the cell suspension on a shaking platform or rotating mixer at a slow setting for 10–20 min at room temperature.
4. Remove insoluble cell debris by centrifugation at 16,000 × g for 20 min at 4°C.
5. Transfer the supernatant to a fresh tube. For SDS-PAGE analysis, remove a small sample (25–50 µl) and combine with equal volume of 4X SDS Sample Buffer (Cat. No. 70607-3). Immediately heat for 3 min at 85°C to denature proteins and then store at –20°C until SDS-PAGE analysis.