xTractor™ Buffer Bacterial Sample Preparation Protocol-At-A-Glance (PT3735-2)

Please read the User Manual before using this Protocol-at-a-Glance. This abbreviated protocol is provided for your convenience, but is not intended for first-time users.

This procedure has been optimized for extraction of native proteins from fresh or frozen bacterial cell pellets using xTractor Buffer. The volumes of this extraction can be adjusted, as long as 20 ml of xTractor Buffer are used per 1 g of cell pellet.

1. Add 20 ml of xTractor Buffer to 1 g of bacterial cell pellet. Mix gently. Pipet the mixture up and down to fully resuspend the pellet.

   NOTE: For a ~1 g bacterial pellet, we harvested approximately 500 ml of *E. coli* culture, which had been induced at log phase (O.D. = 0.6–0.8) and then incubated an additional 2–4 hr for protein production.

2. [OPTIONAL]: Add 40 µl of 5 U/µl DNase I solution and 200 µl of 100X Lysozyme solution.

   NOTES:

   - DNase I reduces the viscosity of the lysate, allowing for more efficient removal of cellular debris. DNase can be used without lysozyme. However, if you are you are treating cells with lysozyme, then you must treat cells with DNase I as well.
   - Lysozyme helps to fully disrupt bacterial walls, and thus it has been demonstrated to be highly beneficial in extraction of high molecular weight proteins (>40 kDa). However, lysozyme should be omitted for mammalian extraction procedures as well as when lysozyme interferes with your protein's functionality.
   - The Lysozyme solution might form a precipitate. Resuspend the contents of the bottle and apply 200 µl of suspension directly to the mix or (optionally) centrifuge 200 µl of Lysozyme solution for 5 min at 14,000 RPM, and use the supernatant for the lysis.

3. Mix gently, pipetting up and down several times.

4. Incubate with gentle shaking or stirring for 10 min at room temperature. (You may incubate the solution at 4°C, if desired).

   NOTE: At the end of this incubation period, there should be no visible particles. If cell pellet fragments are present, resuspend them by pipetting the solution up and down and incubating for an additional 1–2 min.

5. The resulting cell lysate can now be applied directly to a TALON® CellThru Column, or the lysate supernatant can be applied to any other TALON or His60 Ni Resin column after centrifuging at 10,000–12,000 x g for 20 min at 4°C.

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