xTractor™ Buffer Baculovirus Sample Preparation Protocol-At-A-Glance

I. Introduction

This protocol is provided for extraction of native proteins from fresh or frozen baculovirus-infected cell pellets **using xTractor Buffer**, a buffer which has been optimized for his-tagged protein extraction and is compatible with all IMAC resins.

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.

II. Protocol: Extracting Proteins from Baculovirus-Infected Cells

This procedure has been optimized for extraction of native proteins from fresh or frozen baculovirus-infected 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. Harvest cell culture

a. Harvest the baculovirus-infected cell culture by centrifugation at 1,000–3,000 x g for 15 min at 4°C. Remove the supernatant.

b. Store cell pellet at -20°C for long-term storage or proceed to the next step.

2. Resuspend the cell pellet

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

3. Optional step – DNase I/Protease inhibitor

Add 40 µl of 5 units/µl DNase I solution, and a protease inhibitor cocktail to prevent protein degradation. We recommend that you use our **ProteoGuard™ EDTA-Free Protease Inhibitor Cocktail** (Cat. Nos. 635672 & 635673). Mix gently, pipetting up and down several times.

NOTE:
- DNase I reduces the viscosity of the lysate, allowing for more efficient removal of cellular debris.

4. Incubation

Incubate with gentle shaking for 10 min at room temperature. (If desired, incubate the solution at 4°C).

NOTES:
- At the end of the 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.
- If using TALON® CellThru Resin, skip the clarification step. Load the supernatant directly onto the resin.

5. Lysate clarification

a. Centrifuge the crude lysate at 10,000–12,000 x g for 20 min at 4°C. Carefully transfer the supernatant to a clean tube without disturbing the pellet.

NOTE: If the supernatant is not clear, centrifuge a second time or filter through a 0.45 µm membrane (e.g., cellulose acetate) to avoid clogging the IMAC column with insoluble material.

b. Store the supernatant on ice until ready to use.

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