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

I. Introduction

This protocol is provided for extraction of native proteins from frozen mammalian 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 Mammalian Cells

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

NOTE: For adherent cells that are 90% confluent, we find that two 150 mm culture plates, when combined, yield ~150 mg of cells. Before starting the freeze-thaw procedure, we wash the cells four times with PBS (20 volumes per wash).

1. Cell preparation

a. Add 50–150 mg of cultured cells to a pre-weighed centrifuge tube. Centrifuge at 1,000–3,000 x g for 5 min at 4°C. Remove the supernatant. Wash with 2 x PBS and decant the supernatant. Aspirate the residual liquid.

b. Centrifuge the tube again at 1,000–3,000 x g for 2 min at 4°C. Aspirate the residual traces of liquid. Weigh the cell pellet.

c. Store the cell pellet at -80°C for long-term storage or freeze the cell pellet in liquid nitrogen and proceed to the next step.

2. Resuspend the cell pellet

Add 20 µl of xTractor Buffer to 1 mg of cell pellet. Mix thoroughly by vortexing until the mixture is homogeneous.

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.

NOTES:

- DNase I reduces the viscosity of the lysate, allowing for more efficient removal of cellular debris.
- DO NOT USE lysozyme as it may interfere with protein function.

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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