

## I. Introduction

This protocol is provided for extraction of native proteins from fresh or frozen yeast 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 Yeast Cells

This procedure has been optimized for extraction of native proteins from fresh or frozen yeast cell pellets using xTractor Buffer. The volumes of this extraction can be adjusted, as long as a 1 to 30 ratio is used (1 ml xTractor Buffer for every 30 ml of original culture volume).

### 1. Prepare yeast cells

- a. Grow cells overnight to log phase. Harvest cells by centrifugation at 700 x g for 5 min at 4°C. Wash with water, centrifuge and remove the supernatant.
- b. Store cell pellet at -20°C or -80°C for long-term storage or proceed to the next step.

### 2. Resuspend the cell pellet

- a. Resuspend cells in the xTractor Buffer with zymolyase (250 Units of Zymolyase 100T per ml of xTractor Buffer) as indicated in Table 1.

**Table 1. Recommended Cell Pellet Resuspension Volumes**

Original Culture Volume	xTractor Buffer with Zymolyase
30 ml	1.0 ml
60 ml	2.0 ml
100 ml	3.3 ml
500 ml	16.7 ml

- b. Add protease inhibitor cocktail to prevent protein degradation. We recommend that you use our ProteoGuard™ EDTA-Free Protease Inhibitor Cocktail (Cat. Nos. 635672 & 635673).
- c. Vortex gently or pipet the mixture up and down until the mixture is homogeneous.

### 3. Incubation

Incubate with gentle shaking for 1 hr at 30°C. At the end of the incubation, remove and place on ice.

### 4. Vortex

Vortex the sample 5 x 30 sec **without** using glass beads, and place the sample on ice for 1 min between each vortexing.

### 5. Lysate clarification

- a. Centrifuge the crude lysate at 20,000 x g for 15 min at 4°C. Carefully transfer the supernatant to a clean tube without disturbing the pellet.
- b. Store the supernatant on ice until ready to use.

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