Clontech Laboratories, Inc.

xTractor™ Buffer & xTractor Buffer Kit User Manual

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Table of Contents

Table of Tables

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I. Introduction

xTractor Buffer has been optimized for his-tagged protein extraction and is compatible with all IMAC resins. For researchers who require lysozyme and DNase I, we also offer the **xTractor Buffer Kit**. xTractor Buffer is based on a mild non-ionic detergent that gently disrupts either bacterial or mammalian cells.

The extraction method is simple. Just resuspend the cell pellet in the buffer and mix gently for 10 minutes. Then centrifuge or filter the lysate and load it on any IMAC resin column to isolate your his-tagged proteins.

II. Product Information

- **xTractor Buffer** can be used for extraction of proteins from bacterial, yeast, baculovirus-infected, or mammalian cells; and from freshly prepared cells or frozen cells. The extraction volumes can be adjusted, as long as 20 ml of xTractor Buffer are used per 1 g of cell pellet.
- xTractor Buffer is compatible with a variety of reagents. If desired, EDTA-free protease inhibitors, salts, denaturing reagents, and reducing reagents can be added directly to this buffer.
- Lysozyme and DNase I can be added to extract high molecular weight proteins that cannot be extracted unless the bacterial cell wall and membranes are completely disrupted. The **xTractor Buffer Kit** contains 200 ml of xTractor buffer, as well as DNase I and lysozyme.
- When extracting high molecular weight proteins from mammalian cells, lysozyme should be omitted, since it may interfere with protein function.
- DNase I reduces the viscosity of the lysate, allowing for more efficient removal of cellular debris. DNase I can be used without lysozyme. However, if you are treating cells with lysozyme, you **must** treat cells with DNase I as well.
- ProteoGuard™ EDTA-Free Protease Inhibitor Cocktail is available separately (Cat. Nos. 635672 & 635673).
- Clarified cell lysates prepared with xTractor Buffer or the xTractor Buffer Kit are compatible with Clontech's TALON® Resins (Cat. Nos. 635501, 635506, 635601, 635650 & 635655), His60 Ni Resins (Cat. Nos. 635657 & 635659–635664), or any other IMAC resins.
- Crude cell lysates which have not been subjected to a clarification step are compatible with TALON CellThru resins (Cat. Nos. 635509 & 635510).

III. Protein Extraction

PLEASE READ THE ENTIRE PROTOCOL BEFORE STARTING.
Follow the appropriate section below, depending on your starting material: bacterial cell culture (Section A), yeast cells (Section B), baculovirus-infected cells (Section C), or mammalian cells (Section D).

A. Extracting Proteins from Bacterial Cell Culture

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

1. Harvest cell culture

Harvest the bacterial cell culture by centrifugation at **1,000–3,000 x g** for **15 min at 4°C**. Remove the supernatant.

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 pellet

NOTE: A log-phase *E. coli* culture (O.D.=0.6–0.8), when induced for 2–4 hours, would be expected to provide ~20–40 mg of bacterial pellet from 2 ml of the culture.

3. Optional step – Lysozyme & DNase I/Protease inhibitor

Add **40 µl of 5 units/µl DNase I solution** and **200 µl of 100X lysozyme solution**. Add **EDTA-free protease inhibitor**. 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. DNase I can be used without lysozyme. However, if you are treating cells with lysozyme, you must also treat these cells with DNase I.
- Lysozyme helps to fully disrupt bacterial walls and is highly beneficial when extracting high molecular weight proteins (>40 kDa).
- The lysozyme solution may 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 & use the supernatant to perform the lysis.
- We recommend that you use our ProteoGuard EDTA-Free Protease Inhibitor Cocktail (Cat. Nos. 635672 & 635673).

4. Incubation

Incubate with gentle shaking for 10 min at room temperature. (If desired, you may 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

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.

Store the supernatant on ice until ready to use.

B. Extracting Proteins from Yeast

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** (1ml xTractor Buffer for every 30 ml of original culture volume).

1. Prepare yeast cells

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.

Store cell pellet at -20°C or -80°C for long-term storage or proceed to the next step.

2. Resuspend the cell pellet

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

Add protease inhibitor cocktail to prevent protein degradation. We recommend that you use our ProteoGuard EDTA-Free Protease Inhibitor Cocktail (Cat. Nos. 635672 & 635673).

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

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

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.

Store the supernatant on ice until ready to use.

C. 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

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

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**. Add **EDTA-free protease inhibitor**. 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.
- We recommend that you use our ProteoGuard EDTA-Free Protease Inhibitor Cocktail (Cat. Nos. 635672 & 635673).

4. Incubation

Incubate with gentle shaking for 10 min at room temperature. (If desired, you may 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

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.

Store the supernatant on ice until ready to use.

D. 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

Add **50–150 mg of cultured cells** to a preweighed 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.

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

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**. Add **EDTA-free protease inhibitor**. 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, you may 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

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.

Store the supernatant on ice until ready to use.

IV. Troubleshooting

Table 2. Troubleshooting Guide for xTractor Buffer and the xTractor Buffer Kit

Description of Problem	Possible Explanation	Solution
Protein is degraded during extraction	Protein of interest is degraded by proteases	Use mild extraction conditions in the presence of protease inhibitors (e.g., ß-ME and EDTA) at 4°C. Be sure to remove EDTA before purifying on IMAC resins.
		Try using more protease inhibitor, e.g., double or triple the inhibitor concentration.
		Work quickly at 4°C to reduce the time for the initial purification step.
Protein of interest is not solubilized	Protein is expressed in inclusion bodies	Add lysozyme and DNase I to xTractor Buffer.
		Use an inclusion body solubilization reagent.
		Adjust expression conditions.
Lysate is still viscous	Incomplete DNA fragmentation	Add more DNase I to the xTractor Buffer and stir on ice for 15 min.
		Sonicate 3-4 times in 20–30 sec bursts, on ice.
Target protein is trapped in the pellet	You are purifying a high molecular weight protein	If purifying from bacterial cells: add lysozyme and DNase I to xTractor Buffer.
		If purifying from mammalian cells: add DNase I (do not add lysozyme).
	You are purifying a membrane-bound protein or multiprotein complexes	Do not clarify the lysate. Use Clontech's TALON CellThru for direct purification from crude cell lysates (unclarified cell lysates).

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This document has been reviewed and approved by the Clontech Quality Assurance Department.