I. Introduction

His60 Ni Magnetic Beads (Cat. No. 635693 & 635692) can be used for microscale purification of his-tagged proteins on a magnetic separator. Procedures are provided here for preparing clarified lysates and purifying proteins under native and denaturing conditions.

II. Buffers & Other Additional Materials Needed

A. Buffers for Extracting & Purifying Proteins

Use the buffers supplied in the **His60 Ni Buffer Set** (Cat. No. 635665) to extract (Section III) and purify (Section IV) proteins under native and denaturing conditions as follows:

1. Buffers for Native Conditions

- **xTractor™ Buffer**: the lysis buffer for protein extraction in Section III.A.
- **His60 Ni Equilibration Buffer:** pH 7.4, ready to use, no dilution needed.
- **His60 Ni Elution Buffer**: pH 7.4, ready to use, no dilution needed.
- **Wash Buffer**: Wash Buffer is easily made by mixing 710 µl of His60 Ni Elution Buffer with 9.29 ml of His60 Ni Equilibration Buffer.

2. Buffers for Denaturing Conditions

- **His60 Ni Equilibration Buffer:** add urea to a final concentration of 8 M, and if necessary, readjust the pH to 7.4. (Use this buffer as the lysis buffer for protein extraction in Section III.B. Sonication of the lysate may be necessary.)
- **His60 Ni Elution Buffer:** add urea to a final concentration of 8 M, and if necessary, readjust the pH to 7.4 after diluting stock.
- **Wash Buffer:** Wash Buffer is easily made by mixing 710 µl of His60 Ni Elution Buffer with 9.29 ml of His60 Ni Equilibration Buffer. Add urea to a final concentration of 8 M, and if necessary, readjust the pH to 7.4 after diluting stock.

B. Additional Materials Required

- **Magnetic separator (colorless or white for best visibility, since the beads are black)** **[**We recommend Clontech's **Magnetic Stand** (Cat. No. 631964)]
- **1.5 ml and 0.5 ml microfuge tubes**
- **DNase I**

NOTE: Although xTractor Buffer is included in the His60 Ni Buffer Set (Cat. No. 635665), it is also available separately in different sizes (Cat. Nos. 635656, 635671 & 635625)—or in the xTractor Buffer Kit (Cat. No. 635623), which also includes DNase I and lysozyme.

III. Protein Extraction Protocols

IMPORTANT: We strongly recommend using **ProteoGuard™ EDTA-Free Protease Inhibitor Cocktail** (Cat. No. 635673) when preparing your protein extract. Add 10 µl of Protease Inhibitor Cocktail per ml of lysis buffer

[xTractor Buffer for native proteins (Section III.A) or **Denaturing** His60 Ni Equilibration Buffer for denatured proteins (Section III.B)] **before** lysing cells to yield a 1X final concentration of inhibitors. For more information, refer to the ProteoGuard Protocol-At-A-Glance (type "ProteoGuard" in the search box at www.clontech.com/manuals).

A. Protocol: Standard Sample Preparation to Isolate Native Proteins

1. Add 0.5 ml of xTractor Buffer per 25 mg of cell pellet. The volume of xTractor Buffer can be increased or decreased depending on the size of the cell pellet.
2. **[Optional]**: Add 1 µl of 1 unit/µl DNase I solution.
3. Mix gently, pipetting up and down several times.
4. Incubate with gentle shaking for 10 min at room temperature or at 4°C, if desired.
5. Centrifuge the sample at 10,000–12,000 x g for 20 min at 4°C to remove any insoluble material.
6. Carefully transfer the supernatant to a clean tube without disturbing the pellet. Set aside a small portion of this clarified sample at 4°C or on ice for protein assays and SDS-PAGE analysis and proceed with the His60 Ni Magnetic Beads purification protocol (Section IV).

B. Protocol: Standard Sample Preparation to Isolate Denatured Proteins

1. Add 0.5 ml of **Denaturing** His60 Ni Equilibration Buffer per 25 mg of cell pellet. The volume of the buffer can be increased or decreased depending on the size of the cell pellet.
2. Gently agitate or stir the sample until it becomes translucent. (Sonication may be necessary to tear open the cell membranes.)
3. Centrifuge the sample at 10,000–12,000 x g for 20 min at 4°C to remove any insoluble material.
4. Carefully transfer the supernatant to a clean tube without disturbing the pellet. Set aside a small portion of this clarified sample at 4°C or on ice for protein assays and SDS-PAGE analysis and proceed with the His60 Ni Magnetic Beads purification protocol (Section IV).

IV. Protein Purification Protocol

A. General Considerations for His60 Ni Magnetic Beads

- Use a pipette to mix buffer thoroughly with the beads to make a homogenous suspension.
- If needed, magnetic beads can be mixed using a vortexer.
- If there is a great deal of liquid/buffer adhering to the sides of the tube, centrifuge the tube using a microfuge before placing it on a magnetic separator.
- During washing and separation procedures, ensure that the beads are adhering to the side of the tube toward the magnet before removing the supernatant.
- His60 Ni Magnetic Beads are for single use only. They cannot be regenerated.

B. Protocol: Protein Purification under Native or Denaturing Conditions

1. Aliquot 100–200 µl of beads into a 1.5 ml microfuge tube.
2. Place the tube on a magnetic separator for 1 min and remove storage buffer.
3. Remove the tube from the magnetic separator and add 0.5 ml of deionized water to the beads.
4. Mix the liquid and the beads thoroughly using a pipette.
5. Place the tube on a magnetic separator and remove the supernatant.
6. To equilibrate the beads, add 0.5 ml of His60 Ni Equilibration Buffer.
7. Repeat steps 4 and 5.
8. Add the cell lysate (from Sections III.A or B) to the beads.

   NOTE: If the cell lysate volume is less than 200 µl, add sufficient His60 Ni Equilibration Buffer to bring the volume up to at least 200 µl. This is necessary to ensure thorough mixing of beads with the cell lysate, for optimal binding.

9. Mix on a rotary shaker for 30 min at room temperature.

   NOTE: If the protein is vulnerable to degradation at room temperature, incubate at 4°C for 1 hr. Clontech's **ProteoGuard EDTA-Free Protease Inhibitor Cocktail** (Cat. No. 635673) can also be added during the incubation.

10. Place on a magnetic separator and collect the supernatant.
11. Remove the tube from the magnetic separator and add 0.5 ml of His60 Ni Equilibration Buffer.
12. Mix thoroughly and let the tube stand for 1 min before placing it on a magnetic separator and collecting the first wash.
13. Repeat Steps 11 and 12 twice to collect the second and third washes, respectively.
14. **[Optional]**: If necessary, repeat Steps 11 and 12 under more stringent conditions using 0.5 ml of Wash Buffer (For Wash Buffer preparation, please see Section II.A).
15. To elute the protein, add 50 µl of Elution Buffer. The volume of Elution Buffer can be varied depending on the amount of beads used. 50 µl of Elution Buffer can be used for eluting from 200 µl of bead suspension. Most of the protein will elute in this fraction. Smaller volumes, such as 25 µl, can be used if a concentrated sample is needed. Volumes below 25 µl may be difficult to handle.

16. Mix for 5 min and collect Eluate 1.
17. Add another 50 µl of Elution Buffer.
18. Mix for 1 min and collect Eluate 2.
19. If necessary, Steps 17 and 18 can be repeated twice to ensure that protein recovery is maximized.
20. Use spectrophotometric and SDS-PAGE analyses to determine which fractions contain the bulk of his-tagged protein.

NOTE: A Bradford protein assay is recommended for measuring protein yields. Since the detergents in the xTractor Buffer may interfere with the Bradford assay, it is advisable to run the original lysate and nonadsorbed fraction at a 1:5 dilution or use a BCA assay for undiluted samples.

V. Troubleshooting Guide

Description of Problem	Possible Explanation	Solution
No protein binds to the beads when using overnight culture.	The pH is not within the correct range.	Check the pH and ensure that it falls between 7–8.
The beads fail to migrate to the magnet.	The solution is too viscous.	1. Add sufficient DNase 1 (1 unit/ml of culture) and mix thoroughly before adding beads. 2. Dilute the sample further with His60 Ni Equilibration Buffer.

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