I. Introduction

TALON Magnetic Beads (Cat. Nos. 635636 & 635637) can be used for microscale purification of his-tagged proteins on a magnetic separator. Procedures are provided here for preparing clarified and crude 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 **TALON Magnetic Beads Buffer Kit** (Cat. No. 635638) 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 Sections III.A & III.C.
- **1X Equilibration/Wash Buffer**: if necessary, readjust the pH to 7.0 after diluting stock.
- **1X Elution Buffer**: if necessary, readjust the pH to 7.0 after diluting stock.

2. Buffers for Denaturing Conditions

- **1X Equilibration/Wash Buffer**: add guanidine-HCl to a final concentration of 6 M, and if necessary, readjust the pH to 7.0 after diluting stock. (Use this buffer as the lysis buffer for protein extraction in Section III.B.)
- **1X Elution Buffer**: add guanidine-HCl to a final concentration of 6 M, and if necessary, readjust the pH to 7.0 after diluting stock.

B. Additional Materials Required

- **Magnetic separator (colorless or white for best visibility, since the beads are black)**
- **1.5 ml and 0.5 ml microfuge tubes**
- **DNase I**

NOTE: Although xTractor Buffer is included in the TALON Magnetic Beads Buffer Kit (Cat. No. 635638), it is also available separately in different sizes (Cat. Nos. 635656, 635671 & 635625)—or in the TALON 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 (Sections III.A & III.C)) or denaturing 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 (type PT5140-2 in the keyword field 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 TALON Magnetic Beads purification protocol (Section IV).

NOTE: The uncentrifuged crude cell lysate can also be applied to TALON Magnetic Beads. However, the lysate may have to be diluted further or require more DNase to decrease the viscosity of the solution.

B. Protocol: Standard Sample Preparation to Isolate Denatured Proteins

1. Add 0.5 ml of **Denaturing** 1X Equilibration/Wash 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.
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 TALON Magnetic Beads purification protocol (Section IV).

NOTE: Samples containing 6 M guanidine must be dialyzed overnight against buffer containing 8M urea before loading on a gel.

C. Protocol: Sample Preparation Directly from Overnight Cultures

1. Dilute overnight culture 1:1 with xTractor Buffer and add DNase to a concentration of 1 unit/ml of culture. (For example, dilute 0.5 ml of an overnight culture with 0.5 ml of xTractor Buffer and add 1 unit of DNase.)
2. Mix thoroughly at 4°C for 30 min.
3. **[Optional]** If the culture is still too viscous, dilute it with sufficient 5X Equilibration/Wash Buffer to obtain a final concentration of 1X Equilibration/Wash Buffer.
4. Check pH to ensure that it falls between 7–8 for optimal binding and proceed with the TALON Magnetic Beads purification protocol (Section IV).

IV. Protein Purification Protocol

A. General Considerations for TALON 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.
- TALON 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 1X Equilibration/Wash Buffer.
7. Repeat steps 4 and 5.
8. Add the cell lysate (from Sections III.A, B, or C) to the beads.

   NOTE: If the cell lysate volume is less than 200 µl, add sufficient 1X Equilibration/Wash 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 1X Equilibration/Wash 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 5–10 mM imidazole in 1X Equilibration/Wash Buffer.
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. In a specific instance, when using 200 µl of bead suspension, 60% of the total protein was eluted in the first 50 µl fraction, 20% in the second, 10% in the third, and 5% in the fourth.
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 5X Equilibration/Wash Buffer to obtain a final concentration of 1X Equilibration/Wash Buffer.

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