Protein extraction and purification based on Thermo Fisher's Protocol

Reagents:

- Pierce™ His Protein Interaction Pull-Down Kit (Thermo Fisher)
    - Catalog: 21277
    - 25 reactions
    - 10-25 mg of protein per mL of resin
    - Content:
        - § Cobalt resin
        - § Lysis buffer
        - § Spin columns
        - § Collection tubes
        - § Stock solution
- Inclusion body solubilization reagent (Catalog number: 78115)
- Ice
- Protease inhibitor coctel

Equipment and additional materials:

- Microcentrifuge
- Ice tray
- Pipettes and tips (2 µL - 1 mL)
- Vortex
- 0.2 µm filter sterilization unit (500 mL capacity)
- 1.5 mL microcentrifuge tubes
- 2 mL collection tubes
- Analytical balance

Procedure

I. *Buffer preparation*

1. Reconstitute TBS pack with 500 mL of ultra pure water.
2. Filter using a 0.2 µm filter and store at 4 °C

II. *Cell lysis*

1. Transfer 5mL of the iduced *E. coli* culture to a sterile centrifuge tube (This quantity can be increased if the protein has low expression levels)
2. Centrifuge at 5000 × *g* for 5 minutes and discard culture supernatant.

3. Resuspend pellet in 1mL of TBS per 5mL of original culture volume. Mix using a pipette or vortex mixer.
4. Transfer 1mL of cell suspension to a 1.5mL microcentrifuge tube.
5. Centrifuge at 5000 × *g* for 5 minutes and discard supernatant.
6. Resuspend pellet in 200µL of ice-cold TBS per 5mL of original culture volume. Mix using a pipette or vortex mixer.

(For optimal results, use protease inhibitor cocktail when preparing cell lysate.=

7. Add 200µL of the Lysis Buffer per 5mL of original culture volume. Immediately invert until thoroughly mixed

(For inclusion body solubilization, use Inclusion Body Solubilization Reagent)

8. Incubate on ice for 30 minutes and periodically invert tubes*****.
9. Centrifuge at 12,000 × *g* for 5 minutes to clarify crude *E. coli* lysate.
10. Decant supernatant to a separate microcentrifuge tube and store on ice. Label this tube "bait lysate."

***In the mean time, start with the next section.**

III. *Protein immobilization*

Cobalt Resin Preparation

1. Label a sufficient number of Spin Columns to include a sample, non-treated resin control and immobilized bait control for each experiment.
2. Prepare a 1:1 wash solution of TBS: Lysis Buffer and add the 4M Imidazole Stock Solution to a final concentration of 10mM imidazole. For each spin column, prepare ~8mL of wash solution and add 20µL of the 4M Imidazole Stock Solution.
3. Resuspend the HisPur Cobalt Resin using a vortex mixer. Pipette 50µL of the slurry into each labeled spin column. For best results, use a cut or wide-bore pipette tip.

(Resin is supplied as a 50% slurry. Settled resin volume per assay is 25µL)

1. Add 400µL of the wash solution to each spin column. Cap both ends of the column and invert several times.
2. Remove both caps and place spin column in a collection tube.
3. Centrifuge at 1250 × *g* for 1 minute. Replace bottom cap. Discard wash solution from collection tube and re-insert spin column.
4. Repeat wash Steps 4 and 5 for a total of 5 washes.

Immobilization of the Protein

1. Apply bottom cap and remove top cap for each Spin Column.
2. Add prepared polyhistidine-tagged fusion protein lysiate to the Spin Column, at least 300µL and replace top cap of each column.
3. Incubate at 4°C for at least 30 minutes with gentle rocking motion on a rotating platform.
4. Remove both caps from each column and place each into a collection tube.
5. Centrifuge at 1250 × *g* for 30 seconds to 1 minute and place on ice.

6. Replace bottom cap on spin column.
1. Add 400µL of wash solution and replace top cap. Invert several times to mix thoroughly.
2. Remove both caps and place spin column in the collection tube.
3. Centrifuge at 1250 × *g* for 30 seconds to 1 minute, discard the wash volume and reuse tube for all wash collections.
4. Repeat wash Steps 7-10 for a total of 5 washes

Protein elution

1. Prepare 1mL of 290mM Imidazole Elution Buffer, by adding 70µL of 4M Imidazole Stock Solution to 930µL of wash solution. Prepare additional wash solution as needed.
2. Thorughly mix Elution Buffer.
3. Apply bottom cap and remove top cap of spin column.
4. Add 250µL of the Elution Buffer to the spin column. Replace top cap to the column.
5. Incubate spin column for 5 minutes with gentle rocking on a rotating platform.
6. Remove both caps and place spin column in a collection tube.
7. Centrifuge at 1250 × *g* for 30 seconds to 1 minute.
8. Perform 3 or 4 elutions per assay so that a complete elution profile can be established for each protein assayed.