Purification of 6x His-tagged Protein (from *E. coli*)

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[Abstract] A polyhistidine-tag is an amino acid motif that contains at least six histidine (His) residues, usually at the N- or C-terminus of the protein. This tag can also be referred to as a hexa histidine-tag or a 6x His-tag. The protocol described here has been developed to purify His-tagged proteins from *E. coli* under denaturing conditions using Ni-NTA agarose beads.

Materials and Reagents

1. Ni-NTA superflow (QIAGEN)
2. Tris base
3. Urea
4. IPTG
5. $NaH_2PO_4 \cdot H_2O$
6. NaOH
7. HCl
8. LB/Amp media
9. Buffer B (see Recipes)
10. Buffer C (see Recipes)
11. Buffer D (see Recipes)
12. Buffer E (see Recipes)

Equipment

1. Centrifuge and rotor JLA8.1000 and JA-20 (Beckman Coulter)
2. Sonicator

Procedure

A. Induction of recombinant proteins

1. Grow 5 ~ 10 ml culture to saturated stage. The next day, inoculate this starter culture in 2 to 4 L of LB/Amp media using 1:50 or 1:100 dilution of saturated culture.
2. Grow the culture till it reaches OD= 0.4 to 0.6. Add IPTG to its final concentration of 0.6 M and induce 6x His-tagged protein production for 4 h.

   Note: The amount of culture required depends on the level at which the protein is expressed, which must be determined empirically for each expression experiment. In a small scale induction experiment, if the expression level is 1.6%, concentration of 6x His-tagged protein ~ 2 mg/L and culture volume is 2 L, then the amount of 6x His-tagged protein is ~ 4 mg.

   Note: 1 mg for antigen production. 1 mg for antibodies affinity purification.
3. Harvest cells using rotor JLA8.1000 at 5,000 rpm for 20 min. Store cell pellets at -80 °C.

B. Preparation of cleared *E. coli* lysates under denaturing conditions

1. Thaw the cell pellet at room temperature (RT) and resuspend in buffer B at 2 ml per gram wet weight.

   The amount of cells required depends on the expression level of the 6x His-tagged protein and the expression system used. The binding capacity of Ni-NTA resins is protein dependent and normally lies between 5-10 mg/ml.
2. Sonicate cells in cold room.

   Setting: Amplitude 30%, 3 min, 15 sec on, 15 sec off. Try 3 min cycle at least twice. Sonication shears genomic DNA, which makes the lysate less sticky.
3. Centrifuge lysate at 10,000 *x g* (11,294 rpm for rotor JA-20) at 8-12 °C to pellet cellular debris.

   Save supernatant. Save 20 µl as input.
4. Proceed to protocols for purification under denaturing conditions.

C. Batch purification of 6x His-tagged proteins from *E. coli* under denaturing conditions

1. Add 1 ml of the pre-washed 50% Ni-NTA slurry to 4 ml lysate and mix gently by rotating for 60 min at RT.

   The amount of lysate required depends on the expression level of the 6x His-tagged protein and the expression system used. The binding capacity of Ni-NTA resins is protein-dependent and normally lies between 5 -10 mg/ml. I use 1 ml resin for 10 ml lysate.
2. Load lysate-resin mixture carefully into an empty column with the bottom cap still attached.
3. Remove the bottom cap and collect the flow through.

   Collect flow through (20 µl) for SDS-PAGE analysis.

4. Wash twice with 4 ml buffer C.
   Keep wash fractions (20 µl) for SDS-PAGE analysis.
5. Elute the recombinant protein 4 times with 0.5 ml buffer D, followed by 4 times with 0.5 ml buffer E. Collect fractions and analyze by SDS-PAGE.
   Monomers generally elute in buffer D, while multimers, aggregates, and proteins with two 6x His tags will generally elute in buffer E.

Notes

The amount of culture required for an experiment like this will depend on the level at which the protein is expressed, which must be determined empirically for each expression experiment.

Recipes

1. Buffer B (1 L)
   100 mM NaH_2PO_4 [13.8 g $NaH_2PO_4 \cdot H_2O$ (MW 137.99 g/mol)]
   10 mM Tris·HCl [1.2 g Tris base (MW 121.1 g/mol)]
   8 M urea 480.5 g (MW 60.06 g/mol)
   Adjust pH to 8.0 using NaOH
2. Buffer C (1 L)
   100 mM NaH_2PO_4 [13.8 g $NaH_2PO_4 \cdot H_2O$ (MW 137.99 g/mol)]
   10 mM Tris·HCl [1.2 g Tris base (MW 121.1 g/mol)]
   8 M urea 480.5 g (MW 60.06 g/mol)
   Adjust pH to 6.3 using HCl.
3. Buffer D (1 L)
   100 mM NaH_2PO_4 [13.8 g $NaH_2PO_4 \cdot H_2O$ (MW 137.99 g/mol)]
   10 mM Tris·HCl [1.2 g Tris base (MW 121.1 g/mol)]
   8 M urea 480.5 g (MW 60.06 g/mol)
   Adjust pH to 5.9 using HCl.
4. Buffer E (1 L)
   100 mM NaH_2PO_4 [13.8 g $NaH_2PO_4 \cdot H_2O$ (MW 137.99 g/mol)]
   10 mM Tris·HCl [1.2 g Tris base (MW 121.1 g/mol)]
   8 M urea 480.5 g (MW 60.06 g/mol)
   Adjust pH to 4.5 using HCl.
   Note: Due to the dissociation of urea, the pH of Buffers B, C, D, and E should be adjusted immediately prior to use. Do not autoclave.

Acknowledgments

This protocol has been modified and adapted in the Espenshade Lab, Johns Hopkins School of Medicine. Funding to support different projects that have used this protocol has come from NIH – National Heart, Lung, and Blood Institute, National Institute of Allergy and Infectious Diseases, the Pancreatic Cancer Action Network, and the American Heart Association.

References

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