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Nickel-NTA Protein Purification

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Protocol status: Working
We use this protocol and it's working

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Abstract

Protocol to purify 6xHis-tagged recombinant proteins expressed in *E. coli* using Ni-NTA pull-down.

Guidelines

Try to perform all steps at 4 ºC as best as possible and keep protein samples on ice. For all spin-concentrate and buffer exchange steps, use slow speed (e.g. 3500-5000 rcf) to prevent precipitation.

Materials

MATERIALS

- Sodium dihydrogen phosphate **P212121**
- Disodium hydrogen phosphate
- Triton-X100
- 1.5 mL Eppendorf tubes
- Glycerol **Bio Basic Inc.** **Catalog #**GB0232.SIZE.500ml
- Poly-Prep Chromatography Columns **Bio-Rad Laboratories** **Catalog #**731-1550
- DTT **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**43816-10ML
- Sodium chloride **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**S3014
- Imidazole **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**I5513
- Bovine Thrombin Protein **Thermo Fisher** **Catalog #**1996000JL
- HisPur™ Ni-NTA Resin **Thermo Fisher** **Catalog #**88221
- Pierce™ HRV 3C Protease Solution Kit (2 units/µL) **Thermo Fisher** **Catalog #**88946
- TEV protease **New England Biolabs** **Catalog #**P8112S
- Tris Hydrochloride (Tris-HCl) **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**RES3098T-B7
- Amicon® Ultra-4 Centrifugal Filter Unit **Merck Millipore (EMD Millipore)** **Catalog #**UFC8003
- Amicon® Ultra-15 Centrifugal Filter Unit **Merck Millipore (EMD Millipore)** **Catalog #**UFC9003

Troubleshooting

Before start

https://www.protocols.io/private/3797D176057F0C37477116D259C75682

Prepare buffers

1h

1 **Buffer recipes** 1h

Resuspension buffer
- 50 mM Tris-HCl, pH 8.0
- 2 mM EDTA

1X Binding buffer
- 50 mM sodium phosphate buffer, pH 8.0 (ensure >1 pH units away from pI of expressed protein)
- 500 mM sodium chloride
- 0.5% Triton X-100
- 10% glycerol
- 10 mM imidazole

N.b. This should reflect the cell lysis buffer (e.g. https://www.protocols.io/private/3797D176057F0C37477116D259C75682).

1X Wash buffer
- 50 mM sodium phosphate buffer, pH 8.0
- 500 mM sodium chloride
- 0.5% Triton X-100
- 10% glycerol
- 20 mM imidazole

1X Elute buffer
- 50 mM sodium phosphate buffer, pH 8.0
- 500 mM sodium chloride
- 0.5% Triton X-100
- 10% glycerol
- 250 mM imidazole

N.b. DTT cleaves/interferes with Ni^{2+}-binding, use low DTT concentrations (<2 mM), in the above buffers, if needed.

Digestion buffer
- 50 mM Tris-HCl, pH 8.0
- 150 mM NaCl

For *storage*, supplement *digestion buffer* with:
- 1 mM DTT
- 20% glycerol

Ni-NTA Affinity Purification

35m

2 Keep solubilised protein samples (from completion of https://www.protocols.io/private/3797D176057F0C37477116D259C75682) on ice to thaw completely. 15m

3 Centrifuge samples (14,000 rcf for 10-20 minutes) and syringe filter (0.45 µm) supernatant into clean microfuge tube. Keep on ice. 30m

4 Add an appropriate amount of Ni-NTA resin slurry (50% slurry in 20% ethanol) into gravity-flow column. For 15 mL bacterial cultures resuspended in 2 mL lysis buffer, equating to ~1-4 mg protein (1-2 mg/mL), 500 µL resin slurry is added.

Top-up column with 5x volume of *1X binding buffer* (e.g. 500 µL slurry = 250 µL resin = ~2 mL 1X binding buffer).

Allow binding buffer to drop into a waste bottle to equilibrate the resin.

5 Transfer syringe filtered soluble protein (i.e. supernatant from step 3) into equilibrated resin, cap column tightly at both ends (including parafilm to prevent leakage) and incubate with shaking for 1-2 hours @ 4ºC (max 10 mL per column). 2h

6 Keep column up-right and allow resin to settle. Once settled, open cap and capture flow-through (FT).

7 Wash resin three-times with *1X Wash buffer* (2x resin volume, i.e. 1 mL) and collect separately (i.e. W1, W2, W3).

8 Elute four-times with *1X Elute buffer* (1X resin volume, i.e. 500 µL), collect seperately (i.e. E1, E2, E3, E4).

9 Run SDS-PAGE of pre-FT (20 µL), FT (20 µL), W1-W3 (20 µL), and E1-E4 (8 µL) to quality-check purification.

10 Store all samples @ 4 ºC for the short-term. Keep columns containing resin for second Ni-NTA step.

Protease digestion

16h

11 Pool elutes containing protein fragment of expected size.

12 Perform buffer exchange via centrifuge filtration (or dialysis) into *Digestion buffer* to reduce [imidazole] < 1mM.

For example, if *1X Elution buffer* contains 250 mM imidazole, you will need 4x centrifugation at a dilution of 1:4 per spin (4 ºC @ 3,500 rcf for 20 minutes).

13 Add appropriate protease (e.g. TEV, Thrombin, HRV 3C) and incubate overnight (or > 16hrs) at 4 ºC. Ensure to add any required cofactors (e.g. citrate for TEV). 16h

14 Equilibrate Ni-NTA resin (from previously used and stored columns) with 10-20 mL *Digestion buffer* and allow resin to settle. Let buffer flow-through.

15 Add digested protein to resin and collect flow-through (dig-FT). Can perform multiple elutes with *Digestion buffer* to obtain as much protein as possible.

16 Run elutes on SDS-PAGE gel to test for cleavage.

17 Pool and concentrate the pure fraction(s) using buffer exchange.

18 Prepare aliquots of purified protein supplemented with 10-20% glycerol and 1mM DTT. Store at -20 ºC.