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Purification of His-Tagged Proteins using Ni-NTA Beads V.1

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Mariángeles vila Maniero¹, Antonella Denise Losinno¹, Karla Vanessa Gaona¹, María eresa Damiani¹

¹Instituto de Bioquímica y Biotecnología, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo

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Karla Vanessa Gaona

Instituto de Bioquímica y Biotecnología, Facultad de Ciencia...

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We use this protocol and it's working

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Abstract

This protocol describes the batch purification of recombinant His-tagged proteins expressed in E. coli. Proteins containing a 6xHis tag are purified using Ni-NTA (Nickel-Nitrilotriacetic Acid) beads. The His-tag allows selective binding to nickel ions, enabling efficient purification under native conditions. After cell lysis, the lysate is incubated with Ni-NTA beads, followed by washing and elution using imidazole.

Guidelines

Ensure all buffers and reagents are freshly prepared. Pre-cool the centrifuge and prepare all materials. It is recommended to streak a sample on an autoinduction plate and a non-induced control plate, in parallel, to assess the specificity and efficiency of the protein expression and purification.

Materials

Lysis buffer: PBS 1X, Triton X-100 1% (v/v). Binding buffer (pH 7.5): Tris 50 mM, NaCl 500 mM, Imidazole 10 mM. Wash buffer (pH 7.5): Tris 50 mM, NaCl 150 mM, Imidazole 300 mM. Elution buffer (pH 7.5): Tris 50 mM, NaCl 150 mM, Imidazole 300 mM. 4X sample buffer: Tris pH 6.8 1M 62.5 mM 1.25 mL, Glycerol 100%, Bromophenol blue 1% 0.04%, SDS 20%, ddH₂O.

Before start

It is recommended to streak a sample on an [autoinduction plate](#) and a non-induced control plate, in parallel, to assess the specificity and efficiency of the protein expression and purification.

Cell Lysis

- 1 Harvest the induced biomass using a sterile microscope slide to gently scrape the bacterial lawn from the induction plate. Resuspend the collected biomass in **12 mL** of lysis buffer.
- 2 Sonicate the suspension using a 650 W ultrasonic processor, applying the following cycle: 1 cycle of 5 minutes with 3 seconds ON and 2 seconds OFF at 70% amplitude. Use a probe with a 6 mm diameter tip (standard for small-volume processing). Perform the sonication in an ice bath to minimize heat generation and protect protein integrity. Repeat until the sample becomes transparent.
- 3 Centrifuge the lysate at **15,000 g** for **15 minutes**.
- 4 Collect the supernatant for purification.

Day 1 – Binding to Ni-NTA Beads

- 5 Take **50 µL** of Ni-NTA bead slurry into a 1.5 mL Eppendorf tube.
- 6 Equilibrate beads with **1 mL** of binding buffer, rotate for 5 min, and centrifuge at **10,000 rpm** for **5 min**. Discard supernatant. Repeat the wash with another 1 mL of binding buffer. Centrifuge again at **10,000 rpm** for **5 min**.
- 7 Add supernatant of sonication to equilibrated beads and incubate overnight at **4°C** in a rotating mixer.

Day 2 – Washing and Elution

- 8 Centrifuge beads at **10,000 rpm** for **5 min** and collect the flowthrough (keep flowthrough on ice).
- 9 Wash beads twice with 5 times the volume of beads (e.g., 250 µL), rotating for **5 min** and centrifuging at **10,000 rpm** for **5 min** each time. Keep washes on ice.
- 10 Elute the protein with elution buffer adding 6 times the volume of beads (e.g., 300 µL), rotate **5 min** and centrifuge at **10,000 rpm** for **5 min**. Collect the eluate and keep it on ice.



- 11 Repeat step 3 for a second fraction.

Bead Cleaning and Storage

- 12 Wash beads three times with distilled water (1 hour on rotator, centrifuge and discard supernatant each time).
- 13 Add the same volume of beads using **20%** ethanol.
- 14 Store the beads at **4°C**.

SDS-PAGE Sample Preparation

- 15 Mix samples with **4X sample buffer** as follows:
 - Flowthrough: 5 µL sample + 5 µL H₂O + 3.3 µL SB 4X
 - Washes and Elutions: 10 µL sample + 3.3 µL SB 4X
 - Boil samples at 95°C for 10 minutes.
- 16 Run on polyacrylamide gel and analyze expression and purification results. Use a resolving gel with an acrylamide concentration between **10-15%**, depending on the molecular weight of the target protein and a 4% stacking gel. Include samples from both autoinduced and non-induced cultures to properly evaluate the specificity and success of the expression and purification process.

Applications

- 17 This general purification protocol using Ni-NTA beads has been successfully applied to purify the following proteins:
 - BST LF mCherry protein
 - Open Vent mCherry protein
 - PFU DNA polymerase

Results

- 18 To illustrate the results of the purification process, the following SDS-PAGE image shows the elution fractions obtained from the OpenVent-mCherry protein purification. A band

appears around 118 kDa, which corresponds to the expected molecular weight of the OpenVent DNA polymerase fused to mCherry

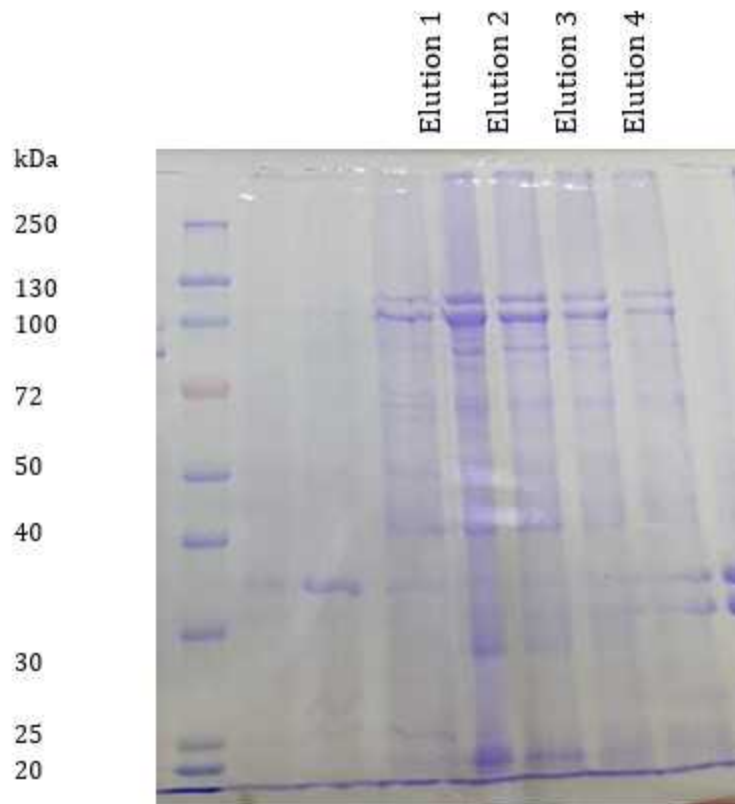


Figure 1. SDS-PAGE analysis of purified OpenVent-mCherry protein.