May 30, 2025 Version 2

Purification of His-Tagged Proteins using Ni-NTA Beads V.2

DOI
https://dx.doi.org/10.17504/protocols.io.kqdg3wz8ev25/v2

Mariángeles vila Maniero[1], Antonella Denise Losinno[1], Karla Vanessa Gaona[1], María eresa Damiani[1]

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

Karla Vanessa Gaona
Instituto de Bioquímica y Biotecnología, Facultad de Ciencia...

DOI: https://dx.doi.org/10.17504/protocols.io.kqdg3wz8ev25/v2

Protocol Citation: Mariángeles vila Maniero, Antonella Denise Losinno, Karla Vanessa Gaona, María eresa Damiani 2025. Purification of His-Tagged Proteins using Ni-NTA Beads. **protocols.io** https://dx.doi.org/10.17504/protocols.io.kqdg3wz8ev25/v2Version created by **Karla Vanessa Gaona**

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

Created: May 30, 2025

Last Modified: May 30, 2025

Protocol Integer ID: 219197

Keywords: tagged protein, purification, enabling efficient purification, efficient purification under native condition, batch purification, purification of hi, selective binding to nickel ion, protein, nta beads this protocol, using ni, nickel ion, nta bead, nitrilotriacetic acid, tag, cell lysi, using imidazole

Funders Acknowledgements:
Chan Zuckerberg Initiative

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

- PBS (Phosphate Buffered Saline)
- Triton X-100
- Tris
- NaCl
- Imidazole
- Glycerol
- Bromophenol blue
- SDS
- ddH_2O

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.

Component	Final concentration
PBS	1X
Triton X-100	1% (v/v)

Table 1. *Lysis buffer composition*

2 Sonicate the suspension using a 650 W ultrasonic processor, applying the following cycle: 1 cycle of 5 minutes with 3 seconds ON and 4 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**.

Component	Final concentration
Tris,	50 mM
NaCl	500 mM
Imidazole	10 mM

Table 2. *Binding buffer composition (pH 7.5)*

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.

Component	Final concentration
Tris,	50 mM
NaCl	150 mM
Imidazole	300 mM

Table 3. *Wash buffer composition (pH 7.5)*

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.

Component	Final concentration
Tris,	50 mM
NaCl	150 mM
Imidazole	300 mM

Table 4. *Elution buffer composition (pH 7.5)*

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

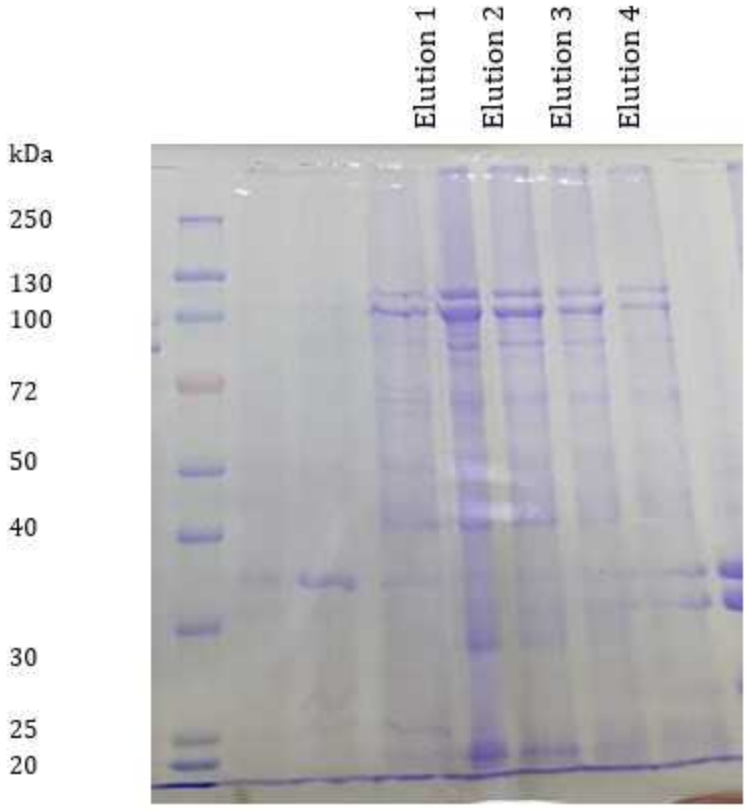

Figure 1. *SDS-PAGE analysis of purified OpenVent-mCherry protein.*