

IMAC purification of His-tagged proteins

Immobilized-metal affinity chromatography (IMAC) captures recombinant proteins carrying a poly-histidine tag (His₆ or His₁₀) on Ni²⁺ chelated to a nitrilotriacetic-acid (NTA) matrix. Imidazole competes for the metal at low concentrations to suppress non-specific binding during washes, and at high concentrations to elute the target.

Small- to mid-scale expression cultures (0.1–1.0 L) are typically purified by gravity flow (*Alternative A*). For analytical screening of expression conditions or protein variants, *Alternative B* describes a spin-column variant. Large-scale cultures (2–4 L) are best purified over an FPLC gradient (*Alternative C*). When the target partitions into inclusion bodies or the poly-histidine tag is not surface-exposed, IMAC can be conducted even under harsh denaturing conditions (*Alternative D*). After any route, protease cleavage and an optional reverse-IMAC pass remove affinity tags and any auxiliary domains.

Buffer pH, ionic strength, and additives in this protocol must be tuned to the protein's stability profile.

Risk assessment

– Nickel(II) sulfate is a KNOWN CARCINOGEN, REPRODUCTIVE TOXIN, and sensitizer – Imidazole is a REPRODUCTIVE TOXIN – Guanidine hydrochloride forms TOXIC GASES with bleach ▷ Weigh nickel(II) sulfate in a certified chemical fume hood ▷ Wear cryo-gloves for liquid-nitrogen handling ▷ Wear gloves, safety glasses, lab coat □ Collect nickel-containing solutions as HEAVY METAL WASTE □ Collect concentrated imidazole and guanidine as HAZARDOUS WASTE □ DO NOT wash into sewer	 In the event of EXPOSURE: ▷ Flush affected eye(s) for 15 min ▷ Warm cryogenic burns with tepid (not hot) water; do not rub Report to: • Principal Investigator/Supervisor Reviewed: May 24, 2026
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Procedures

» Purification from a bacterial host

☐ Bacterial lysis buffer, pH 8.0 ⚡ R0210	☐ 500 mM TCEP (<i>optional</i>)
☐ B-PER Bacterial Protein Extraction Reagent	☐ Benzonase
☐ 100 × Protease inhibitor cocktail (HALT), EDTA-free ⚡ R0072	– or: DNase I
☐ 100 mM Phenylmethylsulfonyl fluoride ⚡ R0035	☐ 1 M Magnesium chloride (<i>optional</i>) ⚡ R0031
☐ 1 500 U/μL Lysozyme (<i>optional</i>) ⚡ R0079	

(1.) Recombinantly express the His-tagged protein of interest in a suitable host strain:

- By default, use *E. coli* BL21(DE3) ⓘ SOP 0034.

This is why: BL21 lacks the Lon and OmpT proteases.

- For transgenes carrying rare bacterial codons (typically from cDNA libraries or unoptimized sources), use *E. coli* Rosetta or Rosetta 2.

Critical: Rosetta strains carry the rare-codon tRNAs on a chloramphenicol-resistance plasmid; maintain selection.

(2.) Harvest the cells by centrifugation at 4 000–6 000 × g for 15–20 min at 4 °C; discard the supernatant.

Hint: Typical pellet mass: 4–5 g per 1 L rich-medium culture; 50 mg per 10 mL small-scale culture.

(3.) *Optional:* Snap-freeze the pellet in liquid nitrogen and store at –80 °C (stable for months).

- (4.) Supplement the lysis buffer with 1 × EDTA-free protease inhibitor (or, failing that, PMSF to 1 mM) and 1 mM TCEP for oxidation-sensitive proteins.

Critical: Keep EDTA out of lysis, binding, and wash buffers, and do not exceed 1 mM DTT or TCEP, or 10 mM β-mercaptoethanol; EDTA strips Ni²⁺ from the resin and reductants reduce it.

Hint: For proteins used in phosphate-sensitive downstream assays (e.g., kinases), use 50 mM Tris-Cl pH 8.0.

- (5.) Lyse the pellet by one of the following methods, depending on culture scale and equipment available:
- For 10–50 mL cultures, resuspend in 4 mL B-PER per 1 g wet cell mass. Transfer to a 1.5 mL tube, and shake on a tabletop shaker at 1 400 rpm for 30 min at 4 °C.
 - For mid-to-large scale cultures, resuspend in 5–10 mL cold bacterial lysis buffer per 1 g wet pellet. Disrupt the cells on ice using a sonic dismembrator with a 0.125 in horn, alternating 20 s pulses at 30–40% amplitude with 40 s rest for a total duty time of 3–6 min.
 - When no sonicator is available, resuspend as above and supplement lysozyme to 1 mg/mL (20 μL stock per 1 mL). Shake at 4 °C for 45 min, then freeze-thaw twice (–80 °C freezer, 4 °C water-bath) to complete lysis.

» Clarification of the lysate

- (1.) Clarify the lysate by centrifugation at 16 000 × g for 30–45 min at 4 °C.
- (2.) Separate the supernatant from the pellet, avoiding the loose lipid layer above the pellet. Retain 20 μL of the supernatant as the load sample.
- (3.) Determine whether the protein of interest is enriched in the supernatant or the pellet by SDS-PAGE  SOP0007. If the target predominantly shows in the pellet (inclusion bodies), proceed via *Alternative D* instead of native capture (*Alternative A–C*).

A > Native batch binding, wash, and elution by gravity flow

- | | |
|--|--|
| ☐ Polypropylene gravity-flow column, with bottom cap and frit | ☐ 6 M Imidazole  R0027 |
| ☐ End-over-end nutator | ☐ Ni-NTA agarose |
| – or: Orbital shaker | |

- (1.) Equilibrate the Ni-NTA agarose:
 - Pipette 50 μL of 50% slurry per 10 mL culture into a conical tube (5.0 mL for 1 L); for low-expressing targets, reduce to 0.5–1.0 mL per 1 L.
 - Let the resin settle. The settled bed volume is 1 vol for the remainder of this protocol.
 - Aspirate the ethanol storage solution.
 - Wash twice with 5 vol cold binding buffer (lysis buffer supplemented with 10 mM imidazole). Let the resin settle and aspirate the supernatant between washes.
- (2.) Pass the clarified lysate through a 0.45 μm low-binding (cellulose acetate) syringe filter. 
- (3.) Add the clarified, filtered lysate to the equilibrated resin and supplement with imidazole to 10 mM (17 μL 6 M imidazole per 10 mL lysate). Nutate at 4 °C for 1 h. 
- (4.) Transfer the resin slurry to the polypropylene gravity column. Allow the flow-through to drain by gravity into a labelled tube. Retain 20 μL of the flow-through for analysis.
- (5.) Wash with 10 vol binding buffer containing 10 mM imidazole by gravity. Retain 20 μL for analysis.
- (6.) Wash with 10 vol binding buffer containing 20 mM imidazole by gravity. Retain 20 μL for analysis.

- (7.) Wash with 5 vol binding buffer containing 50 mM imidazole by gravity. Retain 20 μ L for analysis.

This is why: Skipping the 50 mM wash typically leaves contaminants at 30–65 kDa in the eluate.

- (8.) Transfer the column to a clean collection tube. Elute the bound target with 3–5 vol binding buffer supplemented with imidazole to 500 mM final (833 μ L 6 M imidazole in 10 mL final volume). Collect 1 vol fractions.

Hint: For tightly bound targets (His₁₀ or dimeric tags), hold the first elution volume on the column for 10 min at room temperature before draining, or elute at 750 mM imidazole. For weakly bound targets that bleed during the 50 mM wash, elute at 250 mM imidazole.

B > Spin-column variant for screening scale

☐ Ultrafree-MC HV spin filter, 0.45 μ m

- Use for same-day expression screening at 10–50 mL culture scale.
- (1.) Equilibrate the Ni-NTA in bulk: transfer 10 μ L slurry per 1 mL culture, plus 20% overhead, to one tube. Wash twice with 10 vol binding buffer containing 10 mM imidazole (invert, spin briefly at 2 000 $\times$ g, aspirate), then resuspend to the original slurry volume.
- Hint:* Where the resin volume is too small to pipette, use pre-packed Ni-NTA spin columns at the vendor's volumes.
- (2.) Supplement the clarified lysate with imidazole to 10 mM (dilute the stock to 1 M and add 10 μ L per 1 mL lysate) and add 10 μ L equilibrated slurry per 1 mL culture in a fresh tube. Bind by end-over-end rotation for 1 h at 4 °C.
- (3.) Transfer the slurry to a 0.45 μ m spin filter seated in a collection tube; load in portions if the volume exceeds the filter. Spin 1 min at 2 000 $\times$ g. Retain the flow-through for SDS-PAGE.
- (4.) Wash the retained resin in-filter with 2 vol (at least 200 μ L) binding buffer containing 20 mM imidazole. Spin 1 min at 2 000 $\times$ g.
- (5.) Transfer the filter to a fresh collection tube. Elute twice with 1 vol binding buffer containing 500 mM imidazole, spinning 1 min at 2 000 $\times$ g each time; pool the eluates.

C > FPLC variant (ÄKTA HisTrap FF, preparative scale)

☐ ÄKTA FPLC system, UV detection, conductivity detection, fraction collection

☐ HisTrap FF

- or: HisTrap FF crude, Cytiva
- or: HisTrap FF, 1 mL, Cytiva

- Use for 2–4 L cultures or above.
 - Use when a gradient is needed to resolve the target from a close-eluting contaminant.
- (1.) Equilibrate the column with 5 vol binding buffer containing 10 mM imidazole at 1 vol/min, at 4 °C.
- Hint:* On the 5 mL column, 1 vol/min is 5 mL/min and 0.4 vol is 2 mL.
- (2.) *Critical:* Supplement the lysate with imidazole to 10 mM and filter through a 0.45 μ m filter.
- (3.) Load the cleared lysate at 0.4 vol/min, collecting the flow-through into a labelled fraction.
- (4.) Wash with at least 10 vol binding buffer at 1 vol/min, until A280 returns to baseline.
- (5.) Elute with a four-segment gradient from binding buffer containing 10 mM imidazole (0% B) to 500 mM imidazole (100% B) at 1 vol/min, collecting 0.4 vol fractions throughout:

Start (% B)	End (% B)	Length
0%	10%	2 vol
10%	50%	15 vol
50%	100%	1 vol
100%	100%	5 vol

This is why: Most targets elute in the 10–50% segment, at 150–250 mM imidazole; adjust this segment per target.

D > Denaturing IMAC for inclusion-body targets

- ☐ 10% Triton™ X-100 (optional)  R0057
- ☐ Denaturing urea buffer, pH 7.5
- ☐ Denaturing guanidine buffer, pH 7.5

- Use when the target partitions into the insoluble inclusion-body fraction.
- (1.) *Optional:* Wash the pellet twice in lysis buffer supplemented with 1% Triton™ X-100 to remove membrane debris, then once in detergent-free lysis buffer, each time in the lysis volume and pelleting as for clarification. 
- (2.) Resuspend the pellet in 20 mL denaturing buffer per 1 L culture. Choose the chaotrope:
 - ☐ Use guanidine for dense or hydrophobic inclusion bodies.

Hint: Guanidine solubilizes best but refolds less efficiently. Switch to urea on the column before elution if needed.
 - ☐ Use urea for refolding-sensitive targets and any target with disulfides.
- (3.) Nutate at room temperature for at least 2 h.  2 h
- (4.) Clear the extract by centrifugation at 16 000 × g for 30 min at room temperature.
- (5.) Equilibrate 3–5 mL Ni-NTA slurry per 1 L culture in 5 vol denaturing buffer with 20 mM imidazole.
- (6.) Batch-bind the cleared extract, supplemented with imidazole to 20 mM, for 1 h at room temperature.  1 h
- (7.) Transfer to a polypropylene gravity column. Wash with 10 vol denaturing buffer at 20 mM imidazole.
- (8.) *Optional:* To elute a guanidine-bound target in urea, wash once with 5 vol denaturing urea buffer containing 20 mM imidazole. 
- (9.) Elute with 3–5 vol denaturing buffer containing 250 mM imidazole. Collect 1 vol fractions.

>> Dialysis

- ☐ 5 × Dialysis buffer, pH 7.5  R0214
- ☐ Dialysis tubing
 - or: Dialysis cassette

- (1.) Score the load, flow-through, wash, and elution fractions by SDS-PAGE  SOP0007 and Coomassie staining  SOP0010. Combine elution fractions that show a single dominant band at the expected mass and discard fractions dominated by contaminants.

Hint: Dilute guanidine eluates at least 5-fold with water before adding SDS loading dye; guanidine precipitates SDS. Heat urea eluates to at most 60 °C for less than 5 min; boiling carbamylates the protein.
- (2.) *Critical:* Compute the target protein's isoelectric point and extinction coefficient at 280 nm (ExPASy ProtParam or equivalent). Confirm the dialysis buffer is at least one pH unit away from the pI. 

Resource: ProtParam (<https://web.expasy.org/protparam/>) computes both from the sequence. 
- (3.) *Optional:* If the construct carries a removable affinity tag joined by a protease recognition site, add the protease to the dialysis bag at the lot-specific ratio (see  SOP0044 for TEV). 

Hint: Common proteases stay active in 500 mM imidazole; conditions improve as dialysis proceeds.

- (4.) Transfer the pooled eluate to pre-rinsed dialysis tubing or dialysis cassette. ✕
 - Native eluates: Dialyze against dialysis buffer (diluted to 1×) at a sample-to-buffer ratio of at least 1 : 100 for 3 h at 4 °C.
 - Urea eluates: Dialyze 3 h against 4 M urea in dialysis buffer, then overnight against 2 M urea; the exchange in the next step is into urea-free dialysis buffer.
 - Guanidine eluates: Dialyze robust proteins directly against dialysis buffer; dialyze sensitive proteins against 4 M urea first, then proceed as for urea eluates.
- (5.) Exchange the buffer once and continue dialysis overnight.

Hint: The second bath brings imidazole below 1 mM.
- (6.) Recover the dialysate. Centrifuge at 20 000 × g for 15 min at 4 °C to pellet any precipitate formed during the buffer exchange. Transfer the cleared supernatant to a fresh tube.

⊕ **Optional: Tag removal by reverse IMAC**

- (1.) Confirm by SDS-PAGE that the tagged precursor band is gone.

Hint: To save a day, run this procedure between the 3 h and the overnight dialysis once cleavage is complete; the overnight dialysis then removes the wash imidazole and replaces the final dialysis of this procedure.
- (2.) Equilibrate 0.5–1.0 vol fresh Ni-NTA with 1 × dialysis buffer containing 10–20 mM imidazole.

Critical: 10–20 mM imidazole keeps the tag-free target from binding through surface histidines; higher imidazole lets the His-tagged protease and the freed tag leak through. ←
- (3.) Supplement the dialysate with imidazole to 10–20 mM. Apply it by gravity and collect the flow-through, which contains the cleaved target.
- (4.) Wash with 2.0 vol dialysis buffer containing 10–20 mM imidazole.
- (5.) Return the combined flow-through and washes to fresh dialysis tubing. Dialyze at a sample-to-buffer ratio of at least 1 : 100 for 3 h at 4 °C. ✕

» **Concentration and storage**

- | | |
|---|---|
| ☐ Storage buffer, pH 7.5 | ☐ Ultrafiltration device (<i>optional</i>) |
|---|---|

- (1.) *Optional:* Concentrate the cleared dialysate over an ultrafiltration device. ⊕
- (2.) Exchange the dialysate into storage buffer by brief dialysis or a spin desalting device.

Hint: For a final glycerol below 50%, prepare the storage buffer with twice the desired glycerol at 1 × salt and mix it with an equal volume of dialysate instead; adding neat glycerol would dilute the buffer.
- (3.) Aliquot into single-use (50–200 μL) portions, snap-freeze in liquid nitrogen, and store at –80 °C. ✕

Ni-NTA regeneration and recharge

- | | |
|--|--|
| ☐ Regeneration buffer R0241 | ☐ 20% Sodium dodecyl sulfate R0047 |
| ☐ EDTA strip solution, pH 8.0 | ☐ 100% Ethanol |
| ☐ Nickel recharge solution R0242 | |

- Regenerate between different targets; the same target tolerates up to five runs.
 - Regenerate when the resin turns from light blue to brown-grey.
- (1.) *Optional:* Keep used resin in 20% ethanol at 4 °C until regeneration. ⊕
 - (2.) Transfer the used resin to a gravity column and note the bed volume (this is 1 vol).

- (3.) Pass the following solutions through the column in order, left block first:

Volume	Strength	Solution	Volume	Strength	Solution
2 vol		Water	1 vol each	75%, 50%, 25%	Ethanol
2 vol		Regeneration buffer	2 vol		Water
5 vol		Water	5 vol		EDTA strip solution
3 vol	2%	SDS in water	5 vol		Water
1 vol		Water	3 vol		Nickel recharge solution
1 vol each	25%, 50%, 75%	Ethanol	2 vol		Water
5 vol	100%	Ethanol	3 vol	20%	Ethanol

Hint: The bed turns white on the EDTA strip and light blue on recharge. For heavily fouled resin, add 1 vol of 100% ethanol or leave the column standing in it overnight at 4 °C. To strip loosely bound Ni²⁺ after recharge, add 1 vol regeneration buffer and 2 vol water before the final ethanol.

This is why: The stepwise ramp keeps the agarose bed from cracking; 100% ethanol removes lipids and other hydrophobic contaminants.

- (4.) Store at 4 °C in 20% ethanol. Do not allow the bed to dry out.

Hint: A full strip and recharge restores capacity close to that of new resin. Some fouling accumulates over cycles, but planning for 5–10 mg protein capacity per 1 mL settled resin against 50 mg nominal leaves a wide margin.

Analyses

- Quantify the yield. Measure A280 before the exchange into storage buffer, against dialysis buffer as blank, and convert to concentration using the target's computed ϵ_{280} . For targets lacking Trp/Tyr, quantify by Bradford or BCA against a BSA standard.

Quality assurance: Typical yields are 5–30 mg per 1 L rich-medium culture for well-expressed soluble targets and below 1 mg for difficult ones. Below 0.5 mg per 1 L, revisit expression rather than purification.

Critical: After tag removal, recompute ϵ_{280} on the cleaved sequence.

- Assess purity on a thawed storage aliquot. Resolve 2–5 μg per lane on SDS-PAGE [\[SOP0007\]](#) and stain with Coomassie [\[SOP0010\]](#), or run a microfluidic protein analyzer (e.g. PerkinElmer LabChip). The target should be the dominant band at the expected mass.

Quality assurance: Accept up to 5% contaminants by densitometry for biochemical assays and less than 1% for biophysical and structural work.

- *Optional:* Confirm identity by intact-mass LC-ESI-MS. Do this after protease cleavage and for any preparation destined for structural characterization.

Hint: Look for N-terminal gluconoylation (+178 Da), methionine oxidation (+16 Da per Met), and incomplete cleavage.

- *Optional:* Confirm monodispersity by analytical SEC over a Superdex 200 Increase 5/150 or 10/300 column equilibrated in storage buffer without glycerol. Inject 20–50 μg . The target should elute as a sharp, symmetric peak at the volume expected for its native oligomeric state.

Quality assurance: Aim for less than 5% aggregate (void-volume peak area) for biophysical or structural work and less than 20% for activity assays.

- *Optional:* Run a target-specific activity assay on a thawed storage aliquot. Establish a per-batch specific activity (units per 1 mg) as the reference for downstream experiments.

Hint: For enzymes, a quick activity check after the first freeze-thaw cycle catches freeze-damage that yield and SDS-PAGE miss.

Materials

Purification from a bacterial host

Bacterial lysis buffer ⚡ R0210, pH 8.0, Store at 4 °C.

B-PER Bacterial Protein Extraction Reagent, Thermo Scientific

Protease inhibitor cocktail (HALT) ⚡ R0072, 100 ×, EDTA-free

Phenylmethylsulfonyl fluoride ⚡ R0035, 100 mM

Lysozyme ⚡ R0079 (*optional*), 1 500 U/μL

TCEP (*optional*), 500 mM

Nuclease for genomic DNA digestion

- **Benzonase**
- *or* **DNase I**

Magnesium chloride ⚡ R0031 (*optional*), 1 M

Native batch binding, wash, and elution by gravity flow

Polypropylene gravity-flow column, QIAGEN, 34964, with bottom cap and frit

End-over-end mixing device, operable in a 4 °C cold room or cabinet

- **End-over-end nutator**
- *or* **Orbital shaker**

Imidazole ⚡ R0027, 6 M

Ni-NTA agarose, QIAGEN, 30210, binds up to 50 mg His-tagged protein per 1 mL settled resin; plan for 5–10 mg from crude lysate

Spin-column variant for screening scale

Ultrafree-MC HV spin filter, Millipore, 0.45 μm

FPLC variant (ÄKTA HisTrap FF, preparative scale)

ÄKTA FPLC system, UV detection, conductivity detection, fraction collection

HisTrap FF column, 5 mL reference column; nominal binding capacity 200 mg His-tagged target per column

- **HisTrap FF**, Cytiva
- *or* **HisTrap FF crude**, Cytiva

Hint: Tolerates particulate-rich lysates; filter anyway.

- *or* **HisTrap FF, 1 mL**, Cytiva

Hint: For screening.

Denaturing IMAC for inclusion-body targets

Triton™ X-100 ⚡ R0057 (*optional*), 10%

Denaturing guanidine buffer, pH 7.5

Denaturing urea buffer, pH 7.5

Dialysis

Dialysis buffer ⚡ R0214, 5 ×, pH 7.5, Store at room temperature.

Dialysis device, MWCO at least two-fold below the target mass

- **Dialysis tubing**
- *or* **Dialysis cassette**

Concentration and storage

Storage buffer, pH 7.5, Store at 4 °C.

Ultrafiltration device (*optional*), MWCO at least two-fold below the target mass

- **Amicon® Ultra Centrifugal Filter**, Millipore

Ni-NTA regeneration and recharge

Regeneration buffer ↗ R0241

EDTA strip solution, pH 8.0

Nickel recharge solution ↗ R0242

Sodium dodecyl sulfate ↗ R0047, 20%

Ethanol, 100%

Recipes

Bacterial lysis buffer, pH 8.0

Amount	Ingredient	Stock	Final
94.7 mL	Sodium phosphate dibasic (Na ₂ HPO ₄), pH 9.1 ↗ R0085	0.5 M	47.4 mM
5.3 mL	Sodium phosphate monobasic (NaH ₂ PO ₄), pH 4.2 ↗ R0086	0.5 M	2.6 mM
60 mL	Sodium chloride (NaCl) ↗ R0046	5 M	300 mM
To 1 L	Water, reagent-grade		

Combine Gomori buffers, no titration needed. Store at 4 °C.

Bacterial lysis buffer

47.4 mM Na₂HPO₄, 2.6 mM NaH₂PO₄,
300 mM NaCl, pH 8.0



No GHS hazards at the indicated concentrations

☐ Flush to drain with excess water

Date: Sign: R0210

Denaturing guanidine buffer, pH 7.5

Amount	Ingredient	Stock	Final
25 mL	Tris hydrochloride (Tris-Cl), pH 8.0 ↗ R0056	1 M	50 mM
286.6 g	Guanidine · HCl [50-01-1]	95.53 g/mol	6 M
To 500 mL	Water, reagent-grade		

Adjust to pH 7.5 with HCl.

Denaturing guanidine buffer

50 mM Tris-Cl, 6 M Guanidine, pH 7.5



WARNING

Skin and serious eye irritation; Acute oral toxicity

☐ Hold for EHS review before disposal

Date: Sign:

Denaturing urea buffer, pH 7.5

Amount	Ingredient	Stock	Final
25 mL	Tris hydrochloride (Tris-Cl), pH 8.0 ↗ R0056	1 M	50 mM
240 g	Urea [57-13-6]	60.06 g/mol	8 M
To 500 mL	Water, reagent-grade		

Adjust to pH 7.5 with HCl. **Critical:** Prepare fresh and do not warm above 30 °C; urea decomposes to cyanate, which carbamylates proteins. Deionize over a mixed-bed resin (e.g., Bio-Rad 501-X8) or add ammonium chloride to 25–50 mM.

Denaturing urea buffer

50 mM Tris-Cl, 8 M Urea, pH 7.5

No GHS hazards at the indicated concentrations

☐ Flush to drain with excess water

Date: Sign:

Dialysis buffer, pH 7.5, 5 ×

Amount	Ingredient	Stock	Final
420 mL	Sodium phosphate dibasic (Na_2HPO_4), pH 9.1 	0.5 M	210 mM
80 mL	Sodium phosphate monobasic (NaH_2PO_4), pH 4.2 	0.5 M	40 mM
300 mL	Sodium chloride (NaCl) 	5 M	1.5 M
To 1 L	Water, reagent-grade		

Combine Gomori buffers, no titration needed. Store at room temperature.

5 × Dialysis buffer

42 mM Na_2HPO_4 , 8 mM NaH_2PO_4 , 300 mM NaCl, pH 7.5 [At 1 × dilution]

No GHS hazards at the indicated concentrations

☐ Flush to drain with excess water

Date: Sign: R0214

Storage buffer, pH 7.5

Amount	Ingredient	Stock	Final
10 mL	Dialysis buffer 	5 ×	1 ×
25 mL	Glycerol, anhydrous [56-81-5]		50%
To 50 mL	Water, reagent-grade		

Store at 4 °C.

Storage buffer

1 × Dialysis buffer, 50% Glycerol, pH 7.5



No GHS hazards at the indicated concentrations

☐ Flush to drain with excess water

Date: Sign:

Regeneration buffer

Amount	Ingredient	Stock	Final
286.6 g	Guanidine · HCl [50-01-1]	95.53 g/mol	6 M
5.7 mL	Acetic acid, glacial [64-19-7]	17.4 M	0.2 M
	Skin corrosion (H314)		
To 500 mL	Water, reagent-grade		

Do not adjust the pH.

Regeneration buffer

6 M Guanidine, 0.2 M Acetic acid



WARNING

Skin and serious eye irritation; Acute oral toxicity

☐ Hold for EHS review before disposal

Date: Sign: R0241

EDTA strip solution, pH 8.0

Amount	Ingredient	Stock	Final
100 mL	EDTA, pH 8.0 	0.5 M	100 mM
To 500 mL	Water, reagent-grade		

EDTA strip solution

pH 8.0



WARNING

Skin and serious eye irritation

☐ Dilute below the discharge limit
☐ Flush to drain with excess water

Date: Sign:

Nickel recharge solution

Amount	Ingredient	Stock	Final
13.14 g	Nickel(II) sulfate · 6 H ₂ O [10101-97-0] Respiratory sensitization (H334); Mutagenicity (H341); Carcinogenicity (H350); Reproductive toxicity (H360); Organ toxicity, repeated exposure (H372)	262.85 g/mol	100 mM
To 500 mL	Water, reagent-grade		

Nickel recharge solution



DANGER

Carcinogenicity; Reproductive toxicity; Skin sensitization; Germ cell mutagenicity; Organ damage through prolonged or repeated exposure; Aquatic toxicity (long-term)

- ☐ Collect as HEAVY METAL WASTE
- ☐ DO NOT wash into sewer

Date: Sign: R0242

Troubleshooting

Purification from a bacterial host

In Step 5:

- Lysate is viscous and stringy; the clarification spin leaves a soft, smeary pellet
 - Add benzonase or DNase I to 5–10 U/mL with 2 mM MgCl₂ and incubate 10 min at room temperature before clarification.

Native batch binding, wash, and elution by gravity flow

In Step 2:

- Lysate clogs the filter
 - Centrifuge the supernatant again, at 40 000 × g if a high-speed rotor is available.

In Step 3:

- Resin turns white or brown-grey during or after lysate addition, instead of staying light blue
 - EDTA stripped the Ni²⁺ (white) or a reductant reduced it (brown-grey). Prepare the buffers again without it and use fresh resin; regenerate the stripped resin.

Dialysis

In Step 1:

- Target present in the flow-through and absent from the elution
 - Check that the buffer is at pH 8.0, then re-bind the flow-through to a larger volume of fresh resin.
- Strong contaminant band at about 27 kDa or 74 kDa in the eluate
 - These are the *E. coli* nickel-binding proteins SlyD (runs at about 27 kDa; 21 kDa calculated) and ArnA (74 kDa). Add a fourth wash at 75 mM imidazole.
 - Reduce the resin volume; excess resin binds more host protein.
 - Switch to Co²⁺-TALON resin, which is more selective but has lower capacity; wash at 5–10 mM imidazole.
 - For chronic problem targets, express in an *E. coli* ΔslyD strain.

In Step 4:

- Heavy precipitate forms during the urea downshift
 - Dialyze each stage 6–8 h, or insert a 3 M urea stage.
 - Add 200 mM arginine to all refolding buffers.
 - Dilute the eluate below 0.5 g/L before refolding.

Tag removal by reverse IMAC

In Step 5:

- Low yield in the flow-through
 - If a strip elution shows cleaved target on the resin, raise the equilibration and wash imidazole to 30 mM.
 - Use a smaller bed (0.25 vol).
 - Use Co²⁺-TALON resin, which binds surface histidines more weakly.

Concentration and storage

In Step 3:

- Thawed aliquot has lost activity although the band is intact
 - Snap-freeze by immersion in liquid nitrogen, and aliquot for single use.
 - If glycerol reduces activity, use 10% trehalose or sucrose instead.

Change log

2026-05-24 Benjamin C. Buchmuller Initial version. Consolidates the Ni-NTA gravity workflow (Daniel Summerer lab) and the Ulp1 production workflow (Tom Muir lab) into a single protocol, ports the HisTrap FF gradient from the TEV protease production protocol, and adds denaturing-IMAC and reverse-IMAC variants.

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