IMAC purification of His-tagged proteins

Immobilized-metal affinity chromatography (IMAC) captures recombinant proteins carrying a poly-histidine tag (His_6 or His_{10}) on Ni^{2+} chelated to a nitrilotriacetic-acid (NTA) matrix. Imidazole competes for the metal at low concentrations to suppress non-specific binding, 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: Nov 3, 2026

Procedures

›› *Purification from a bacterial host*

☐ Bacterial lysis buffer, pH 8.0 ◇ R0210
- *or*: B-PER Bacterial Protein Extraction Reagent

☐ 100 mM PMSF ◇ R0035
- *or*: 100 × Protease inhibitor cocktail (HALT), EDTA-free ◇ R0072

☐ 1 500 U/µL Lysozyme (*optional*) ◇ R0079
☐ Benzonase
- *or*: DNase I

(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.) Retain a sample of the culture for SDS-PAGE before induction and one at harvest. Spin down the volume that contains 0.5 OD mL of cells, resuspend the pellet in 70 µL 1 × SDS-PAGE sample buffer SOP 0007, boil at 95 °C, and load 10 µL per lane.

> ***Hint:*** This is about 50–100 µg of total protein per sample and 7–14 µg per lane, with an overexpressed band well inside the staining range. Taking a fixed OD_{600} of cells makes the two lanes comparable without further normalization.

(3.) Harvest the cells by centrifugation at 4 000 × *g* for 20 min at 4 °C. Discard the supernatant. Record the weight of the wet pellet.

> ***Quality assurance:*** Expect 4–5 g per 1 L rich-medium culture.

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

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

> ***Critical:*** Keep EDTA out of lysis, binding, and wash buffers; they strip Ni^{2+} from the resin. Do not exceed 1 mM DTT or TCEP, or 10 mM β-mercaptoethanol, since they reduce the Ni^{2+}.

> ***Critical:*** When the target is itself a protease, check the inhibitor cocktail composition. Broad cocktails carry E-64 and leupeptin which inactivate cysteine proteases, AEBSF and PMSF inactivate serine proteases.

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

(6.) 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, add 1 mg/mL lysozyme (20 µL stock per 1 mL). Shake at 4 °C for 45 min. 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 for SDS-PAGE.

(3.) Determine whether the protein of interest is in the supernatant or the pellet by SDS-PAGE SOP 0007. If it shows predominantly in the pellet (inclusion bodies), proceed via *Alternative D* instead of native capture methods (*Alternative A* to *Alternative C*).

A › *Native batch binding, wash, and elution by gravity flow*

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

(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 µL 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 µL 6 M imidazole in 10 mL final volume). Collect 1 vol fractions.

> ***Hint:*** For tightly bound targets (His_{10} or dimeric tags), hold the first elution volume on the column for 10 min at room temperature, 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 µm

- Use for same-day expression screening at 10–50 mL culture scale.

(1.) Equilibrate the Ni-NTA in bulk: transfer 10 µL 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 × *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 imidazole to 10 mM (dilute the stock to 1 M and add 10 µL per 1 mL lysate) and add 10 µL 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 µm spin filter seated in a collection tube; load in portions if the volume exceeds the filter. Spin 1 min at 2 000 × *g*. Retain the flow-through for SDS-PAGE.

(4.) Wash the retained resin in-filter with 2 vol (at least 200 µL) binding buffer containing 20 mM imidazole. Spin 1 min at 2 000 × *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 × *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 µm 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 A_{280} 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 within the 10–50% segment, often early in it; adjust this segment per target.

D › *Denaturing IMAC for inclusion-body targets*

- ☐ 10% Triton™ X-100 (*optional*) ◇ R0057
- ☐ Denaturing guanidine buffer, pH 7.5
- ☐ Denaturing urea 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 (at 1 ×) ◇ R0214
- ☐ Dialysis tubing
  - *or*: Dialysis cassette

(1.) Score the load, flow-through, wash, and elution fractions by SDS-PAGE 🗎 SOP 0007 and Coomassie staining 🗎 SOP 0010. 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 SOP 0044 for TEV and SOP 0048 for Ulp1).

> ***Hint:*** Imidazole falls as the dialysis proceeds, so a digest started in the bag meets steadily better conditions. Do not count on activity in the undiluted eluate; see the protease's own protocol for what it tolerates.

(4.) Transfer the pooled eluate to pre-rinsed dialysis tubing or a 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 (*optional*)

(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.

> ***Hint:*** An enzyme lot that will be dosed from repeatedly goes instead to 50% glycerol with its reductant and is stored at −20 °C, where it does not freeze solid and a tube can be resampled.

Ni-NTA regeneration and recharge

- ☐ Regeneration buffer ⟋ R0241
- ☐ EDTA strip solution, pH 8.0
- ☐ Nickel recharge solution ⟋ R0242
- ☐ 20% SDS ⟋ R0047
- ☐ 100% Ethanol

- 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 Ni^{2+} 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. The 100% ethanol wash 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 the bed on the low end of the 5–10 mg per 1 mL range leaves an ample margin.

Analyses

○ Quantify the yield. Measure A_{280} 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 SOP 0007 and stain with Coomassie SOP 0010, 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% contaminants 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% aggregate 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, check activity after the first freeze-thaw cycle.

Troubleshooting

Purification from a bacterial host

In Step 6:

– 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 $MgCl_2$ 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 35 000–50 000 × *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 Ni^{2+} (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). Many recurring contaminants elute at about 55 mM, above what most wash procedures use (Robichon et al., 2011); add a fourth wash at 75 mM imidazole.
  - Reduce the resin volume; excess resin binds more host protein.
  - Switch to Co^{2+}-TALON resin, which is more selective but has lower capacity; wash at 5–10 mM imidazole.
  - If target protein expression cannot be raised, the published answer is the NiCo21(DE3) strain, which expresses SlyD, Can and ArnA with a chitin-binding domain so that a chitin column after the IMAC clears them from the eluate (Robichon et al., 2011). Adopting the strain means adding that column: the tagged contaminants hold nickel more tightly than the untagged, not less. Do not reach for a slyD deletion strain instead; SlyD is a folding chaperone and strains lacking it grow poorly.

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 Co^{2+}-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.

Materials

Purification from a bacterial host

- ☐ **Lysis buffer**
  - **Bacterial lysis buffer**, pH 8.0 ◇ R0210
    Store at 4 °C.
  - *or* **B-PER Bacterial Protein Extraction Reagent**
    Thermo Scientific
- ☐ **Protease inhibitor**
  - **Phenylmethylsulfonyl fluoride (PMSF)**, 100 mM ◇ R0035
  - *or* **Protease inhibitor cocktail (HALT)**, 100 ×, EDTA-free ◇ R0072
- ☐ **Lysozyme**, 1 500 U/µL ◇ R0079 (*optional*)
- ☐ **Nuclease for genomic DNA digestion**
  - **Benzonase**
  - *or* **DNase I**

Native batch binding, wash, and elution by gravity flow

- ☐ **Polypropylene gravity-flow column**, with bottom cap and frit
  QIAGEN, 34964
- ☐ **End-over-end mixing device**
  Hint: Choose a device operable in a 4 °C cold room or cabinet.
  - **End-over-end nutator**
  - *or* **Orbital shaker**
- ☐ **Imidazole**, 6 M ◇ R0027
- ☐ **Ni-NTA agarose**
  QIAGEN, 30210
  Hint: The resin binds 5–10 mg His-tagged protein per 1 mL settled bed, depending on the target; QIAGEN measures 8.0 mg per 1 mL for a 26 kDa protein. Plan the bed on the low end of that range for a crude lysate.

Spin-column variant for screening scale

- ☐ **Ultrafree-MC HV spin filter**, 0.45 µm
  Millipore

FPLC variant (ÄKTA HisTrap FF, preparative scale)

- ☐ **ÄKTA FPLC system**, UV detection, conductivity detection, fraction collection
- ☐ **HisTrap FF column**, 5 mL reference column
  Hint: Nominal binding capacity is 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**, 10% ◇ R0057 (*optional*)
- ☐ **Denaturing guanidine buffer**, pH 7.5
- ☐ **Denaturing urea buffer**, pH 7.5

Dialysis

- ☐ **Dialysis buffer**, 5 ×, pH 7.5 (at 1 ×) ◇ R0214
  Store at room temperature.
- ☐ **Dialysis device**
  Hint: Choose a 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*)
  Hint: Choose a 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 (SDS)**, 20% ◇ R0047
- ☐ **Ethanol**, 100%

Recipes

Bacterial lysis buffer, pH 8.0

Amount	Ingredient		Stock	Final
94.7 mL	Sodium phosphate dibasic (Na_2HPO_4), pH 9.1	R0085	0.5 M	47.4 mM
5.3 mL	Sodium phosphate monobasic (NaH_2PO_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 Na_2HPO_4, 2.6 mM NaH_2PO_4, 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 7.5	R0055		1 M	50 mM
286.6 g	Guanidine · HCl	[50-01-1]	95.53 g/mol		6 M
To 500 mL	Water, reagent-grade				

Do not adjust the pH.

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 7.5	R0055		1 M	50 mM
240 g	Urea	[57-13-6]	60.06 g/mol		8 M
15 mL	Sodium chloride (NaCl)	R0046		5 M	150 mM
To 500 mL	Water, reagent-grade				

Do not adjust the pH. ***Critical:*** Prepare fresh and do not warm above 30 °C; urea decomposes to cyanate, which carbamylates proteins [Bur09]. 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, 150 mM NaCl, pH 7.5

No GHS hazards at the indicated concentrations

☐ Flush to drain with excess water

Date: Sign:

Dialysis buffer, pH 7.5 (at 1×), 5×

Amount	Ingredient		Stock	Final
420 mL	Sodium phosphate dibasic (Na_2HPO_4), pH 9.1	R0085	0.5 M	210 mM
80 mL	Sodium phosphate monobasic (NaH_2PO_4), pH 4.2	R0086	0.5 M	40 mM
300 mL	Sodium chloride (NaCl)	R0046	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 ×) *[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	R0214	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	R0017	0.5 M	100 mM
To 500 mL	Water, reagent-grade			

EDTA strip solution

pH 8.0

No GHS hazards at the indicated concentrations

☐ 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_2O [10101-97-0]	262.85 g/mol	100 mM
	Respiratory sensitization (H334); Mutagenicity (H341); Carcinogenicity (H350); Reproductive toxicity (H360); Organ toxicity, repeated exposure (H372)		
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

List of references

R.R. Burgess, *Methods Enzymol.* **463** 813-820 (2009).

C. Robichon, J. Luo, T.B. Causey, J.S. Benner, and J.C. Samuelson, *Appl. Environ. Microbiol.* **77**(13), 4634-4646 (2011).

Change log

2026-10-19 **Benjamin C. Buchmuller** Version 1, 2026. Consolidates the Ni-NTA gravity workflow (Daniel Summerer lab) and the Ulp1 production workflow (Tom Muir lab) into one protocol, ports the HisTrap FF gradient from the TEV protease production protocol, and adds the denaturing-IMAC and reverse-IMAC variants, the uninduced sample and pellet weight, and culture sampling as a fixed cell equivalent.

2026-11-03 **Benjamin C. Buchmuller** Version 2. The binding capacity of Ni-NTA agarose is 5 to 10 mg per mL settled bed; the 50 mg once given is QIAGEN's Superflow figure. The denaturing urea buffer carries 150 mM sodium chloride. Credits: Robichon et al. (2011) for the SlyD and ArnA bands and the NiCo21(DE3) alternative; Cytiva, "HisTrap FF crude data file", for the capacity and the reductant limits; Burgess (2009) for the mixed-bed deionization and the ammonium chloride, the 30 °C ceiling being the author's.

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