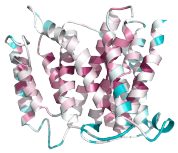
PROVOST & WALLERT RESEARCH

Investigating the Biochemistry &
Cellular Physiology of NHE1
EST. 1998

His Tag Purification

Purification Protocol



Cell lysate Preparation: The key to preparing a lysate (broken cells) is to perform this as quickly as possible while keeping the preparation at low temperature to minimize protease degradation and protein denaturation. A good cell lysate will be clear (no particulate and yellow/tan in color) and not viscous. Viscosity is due to genomic DNA and will cause many problems during chromatography. Pre-Chill buffers, centrifuge heads and other equipment. Keep your solutions and cell pellets IN the ice not ON the ice. For proteins that aggregate or membrane proteins, additional additives include a final concentration of 10% glycerol (aggregation) 0.1% Triton X-100 (aggregation and membrane proteins).

Lysis Instructions:

- Per liter of culture, centrifuge cells and resuspend in 50 ml of His Binding Buffer with the following additions
  - Add Lysozyme (final conc of 1.0-0.5 mg/ml] - Refreeze unused lysozyme).
  - * Optional- use if viscous ***after*** sonication or for larger cultures (>0.5L) DNAseA: 5 ul of 1M $MgCl_2$ per ml of suspension and DNase solution to a final conc of 5-25 ug/ml).
  - Add EDTA free protease inhibitor as per manufacture's instruction. *The half-life is short so do not add until ready*.
  - 10 mM β (2-) mercaptoethanol *** Do not add until prior to use. S*tock conc β ME is 14 M*
- Resuspend pellet with a transfer pipette until the mixture is homogeneous
- Incubate on ice (or in cold room 4°C) for 30 min. Rock or resuspend occasionally.
- Sonnicate ON ICE for 3 x 1-3 min bursts as high as your sample can take without cavitation.
- Centrifuge at 8,000 x g for 20 min at 4°C. NOT in falcon tubes. Use the smaller polypropylene tubes. Must be filled at least halfway to centrifuge at this speed.
- Transfer supernatant to clean 50 ml falcon tube(s). Continue purification.

Column Basics

- 1 liter culture will need 20 ml of beads
- In general, scale the washes and elution using the information below. A rule of thumb is to wash with 10-20 column volumes (volume of packed beads) for the wash buffer. Elute in a total of 5-10 column volumes of elution buffer. Divide the elution into 6-8 total fractions. High expressing proteins may need more elution buffer.

Important Note: A quick check of wash and elution fractions with a bradford assay (20 ul sample mixed with ~1 ml of 1X bradford) will inform you the relative amount of protein in the fraction.

Purification Instructions (per 1000 ml culture adjust to the appropriate volumes as per information above):

- ***Preparation of Ni-Agarose Beads/Resin***:
  - Prepare 20 ml of beads by transferring 40 ml of a 50% slurry of beads equilibrate into a clean column. Wash and equilibrate the column by running 200 ml of His Elution Buffer followed by 400 ml of His Binding Buffer through the column. This SHOULD be done ahead of time! Store prepared beads with a few ml of His Binding buffer at RT.
- **Purification**:
  - Save 100 ul of lysate. Add clarified lysate (if frozen, check for ppt material. If there is any clumpy or ppt material or if the lysate is cloudy, centrifuge and keep the supernatant) to the washed beads.
  - *Batch Binding* - if protein expression is low or the His-tagged protein binds with low affinity then use a batch purification method.
    - Combine the washed beads and lysate onto a drained and capped column or a 50 ml falcon tube for smaller volumes. Use a spatula and/or a transfer pipette to suspend the beads. Tightly cover with parafilm and incubate rocker for 30 min at room temp.
    - Replace the column on the stand and allow most of the beads settle then open column. Add frit back to column. ***Reapply the flow thru***. This is the non-binding protein. Continue with purification
  - *Column Binding* - Flow the clarified lysate through the column. Save the eluate as flow-through in one fraction. If there is a concern with binding efficiency the flow-through can be reapplied. Continue with the purification.
  - Wash beads ~ ***200 ml of Ni-Column His Binding Buffer***. Save as flow through wash in one fraction.
  - Wash the column with ***~300 ml of His Wash Buffer***. *This will remove some of the weakly binding protein. (check flow through for protein with quick bradford). Continue with the His wash buffer until no detectible protein is found in eluate.
  - Elute the protein with ***20 x 15 ml of His Elution Buffer***. Save ALL fractions. Check for eluted protein with quick bradford.
  - Check each fraction for total protein and determine purity by SDS-PAGE – coomasie stain

Quick Bradford. ~1 ml of 1X bradford plus ~20 ul or one drop of eluate or sample. If the dye turns blue-ish there is protein present

His-Binding Buffer:	***His-Wash Buffer:***	***His-Elution Buffer:***
• 50 mM Tris-Cl (pH8.0) • 0.5 mM NaCl • 0.1 mM EDTA	• 50 mM Tris-Cl (pH8.0) • 0.5 mM NaCl • 10 mM Imidazole • 0.1 mM EDTA	• 50 mM Tris-Cl (pH8.0) • 0.5 mM NaCl • 300 mM Imidazole • 0.1 mM EDTA