PROVOST & WALLERT RESEARCH
Investigating the Biochemistry &
Cellular Physiology of NHE1
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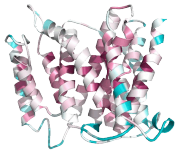
Affinity Chromatography Quick Guide



Overview: There are several steps for each purification. Depending on where you start (freshly plated cells, glycerol stock or transforming cells), this is a three day project plus the time needed to concentrate, dialyze run a gel and perform a protein assay. You will find individual protocols for transformation, expression, lysis, purification, protein assay and SDS PAGE.

Supplies: Cultured bacteria or glycerol stock, LB with antibiotic, IPTG, lysis materials, chromatography resin, buffers, SDS PAGE and protein assay. Check all buffers and materials before starting. Reserve incubator by coordinating with PI. READ background on expression, purification and characterization so you understand the process beyond the steps described in the protocols.

Day 1) Protein Expression: Initial culture - start in the morning unless the culture is a problem then you may need to start early in the day and refrigerate before the culture becomes too dense. (60 min)

Day 2) Expand culture: This will take from 2-3 hours before inducing. Standard 32°C incubation will require 2-4 hours of additional incubation. Otherwise overnight culture at lower temps will take 24 to 48 hours. Centrifuge, rinse pellet and freeze until use unless continuing lysing cells. Freeze pellets

Day 3) Lysis and chromatography: The lysis will take about an hour or little more depending on your preparation. Don't forget the protease inhibitors and DO NOT CONTINUE if the lysate is viscous! The membrane will not centrifuge and your chromatography will take a very long time and not be very successful. This will take a total of 3-4 hours depending on the size of the lysate and chromatography column. The project is not done yet. Leaving your samples in the cold room/refrigerator for more than a day or two can easily result in precipitated or denatured proteins.

Day 4/5) Sample analysis and prep:

- Determine which fraction contains protein by conducting a quick and dirty Bradford assay (500 ul Bradford reagent and 20 ul of sample. Blue=protein).

- Then perform an SDS PAGE on the eluted samples.
  - Depending on your need, you may want to include the lysis and flow through in the first two sample lanes of the gel. To prepare lysate and flow through fraction, mix 5 ul of sample with 50 ul of SDS Sample Buffer and load 10 ul of lysate and 25 ul of flow through. Stain with coomasie.

- Pool the required fractions and dialyze against an appropriate buffer. Buffer choice depends on the next step (another chromatography, using for an experiment or freezing). Alternatively concentrating the sample by ultrafiltration (centricon) and diluting in the new buffer is called buffer exchange and can concentrate the sample. Again the final volume depends on the total protein (size of elution) and later needs. Getting to around 0.5-2.0 mg/ml is a nice concentration. Quick bradford will help you estimate the actual concentration. Remember you may need reducing agents or glycerol to maintain activity of your protein.

- After final prep of your sample you MUST determine the protein concentration. Use a Bradford or BCA assay. BCA is typically used if there are interfering agents in the protein sample.

- Aliquot the protein into multiple fractions. Repeated freeze thaw of samples will cause most proteins to denature. LABEL with the name of the protein, date that correlates with the experiment, protein concentration and the word "protein" and your initials. Freeze in either the -20°C or -80°C freezer. This way we can find the information in your lab book!