PROVOST & WALLERT RESEARCH
Investigating the Biochemistry &
Cellular Physiology of NHE1
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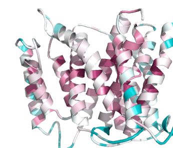
GST Tag Purification

Protocol



GST Purification is an affinity chromatography. GST is a 26 kDa protein expressed in the pGEX plasmid vector. Depending on the vector, many have a thrombin or other protease recognition site. Depending on the protein concentration (typically high levels) GST can dimerize. Using 1-chloro-2,4-dinitrobenze (CNDB) as a substrate, GST activity can be followed using a simple colormetric assay with glutathione.

Protein expression can range from 1 mg to 10 mg per liter of culture. Average yields are 2.5 mg of protein expressed in a 1 liter culture. Binding capacity for most glutathione-sepharose beads is about 25mg per ml of packed beads. The capacity is decreased with higher flow rates. Store at 2oC in 20% ethanol.

Culture Volume	20 liter	4 liters	500 ml	50 ml
Possible Yield	50 mg	10 mg	1.25 mg	0.125 mg
Lysis buffer volume	100 ml	50-100 ml	10-25 ml	2.5 ml
GST column bed volume	10 ml	2 ml	0.5 ml	25 µl
Wash volume (x3) **	100 ml	25 ml	5 ml	250 ml
Elution buffer vol (x5-8) **	10 ml	4 ml	2 ml	

** **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.**

Column Preparation: Wash beads with 10 column volumes of elution buffer followed by 30 column volumes of binding buffer.

Load the column:

- **Batch Load**: 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.
  - Pour the lysate onto a drained and capped column. 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. This the non-binding protein.
- **Conventional load** - run the clarified lysate through the beads. If proteolysis is an issue only do one time through. If the protein poorly binds the beads, run the lysate through the beads twice or use the Batch Load method.

- **Wash the column** with ***GST Wash Buffer***. This will remove some of the weakly binding protein. Continue to wash until there is no protein in eluate (use a quick Bradford analysis).
- **Elute the protein**
  - Add one volume of elution buffer, allow the half of the volume of column (beads) to drain from column, collect as fraction one and CAP/STOP the column (this is called a pulse elution). Incubate the column at room temp for 10 min to elute the fusion protein.
  - Open the column and collect the remaining elution as fraction 2.
  - Continue elution with additional volumes of elution buffer (no need to pulse elute). Save each as a separate fraction. Continue until protein is no longer eluting from the column.

  - Check each fraction for total protein and determine purity by SDS-PAGE – coomasie stain

GST Binding & Wash Buffer:	***GST Elution Buffer:***
• 50 mM Tris-Cl (pH8.0) • 150 mM NaCl • 0.1 mM EDTA	• 50 mM Tris-Cl (pH8.0) • 150 mM NaCl • 0.1 mM EDTA • 10 mM Reduced (Free) glutathione MAKE FRESH avoid oxidized GSH.