

PURIFICATION OF HIS-TAGGED PROTEINS

Materials/Solutions:

Ni-NTA Agarose (QIAGEN)

Lysis Buffer: will vary, but add 0.25 M NaCl before loading on column

Equilibration Buffer: 20 mM Tris, pH 7.5
 0.5 M NaCl

Wash Buffer: 20 mM Tris, pH 7.5
 0.5 M NaCl
 10 mM Imidazole

Elution Buffer: 20 mM Tris, pH 7.5
 0.5 M NaCl
 300 mM Imidazole
 0.03 % Brij-35

Dialysis Buffer (will vary): 50 mM Tris, pH 7.5
 0.1 mM EDTA
 0.1% 2-mercaptoethanol
 50% glycerol

Protocol:

Pour appropriate volume of Ni-agarose resin (use 1-2 mls for a normal expression of up to 10 mg of protein) into a disposable plastic column and equilibrate with several column volumes of Equilibration Buffer to remove the ethanol. Mix resin with precleared lysate in either the column or a 50 ml Falcon tube at 4C on an end-over-end shaker for 1 hour. Adding 0.25 M NaCl to the lysate will decrease nonspecific binding of other proteins.

A quick way to wash the column after binding is to spin it down in a 50 ml Falcon tube at 1000 rpm for 1 min. Pour off the lysate and save as flow-through, then add 30 ml of Wash Buffer, shake and spin down again. Repeat this until the column has been washed 3 times. The 4th time, add 30 ml of Wash Buffer, shake, and pour back into the plastic column. Add a little more Wash Buffer to the Falcon tube to remove the resin that sticks to the side, and pour that on the column as well.

Elute protein with 10 ml of Elution Buffer, adding and collecting 1 ml at a time. After each 1 ml fraction is collected (and placed immediately on ice), do a quick check of the protein concentration by vortexing the fraction and adding 2 ul to 90 ul of Pierce Bradford Reagent (will turn blue if protein is present). This is an easy way to monitor the peak fractions as they come off the column. When done, assay 10 ul each of the peak fractions using the Bradford assay (10 ul fraction + 90 ul water + 1 ml Bradford reagent; measure absorbance at 595 nm and calculate protein concentration based on the standard curve). If the peak fractions contain a high amount of protein, assay 10 ul of a 1:10 or 1:100 dilution to ensure that you're onscale for the Bradford assay.