



NEW YORK CONSORTIUM ON MEMBRANE PROTEIN STRUCTURE

**Midi Scale Purification of Proteins Expressed in the GN Fermenter  
January 2010**

Materials:

Stock Solutions

Resuspension Buffer: 50mM HEPES pH7.8  
300 mM NaCl  
200mM Imidazole pH 7.8  
5% glycerol  
1mM MgCl<sub>2</sub>.

ATP Wash Buffer: 50mM HEPES pH7.8  
300 mM NaCl  
40mM Imidazole pH 7.8  
5% glycerol  
5mM MgCl<sub>2</sub>.

Wash Buffer B: 25mM HEPES pH7.8  
500mM NaCl  
75mM Imidazole pH7.8  
5% glycerol

Elution Buffer: 25mM HEPES pH7.8  
200mM NaCl  
500mM Imidazole pH7.8  
5% glycerol.

Equipment

Robotic Sonicator-Amp 60%  
Glas-Col Plate Shaker  
Roto Shake Genie  
Beckman Coulter Avanti J26-XP centrifuge

Method:

*Resuspension and Lysis*

Remove cell pellets, in VWR 50ml Falcon tubes from -80°C freezer, and resuspend in 25ml Resuspension Buffer w/ 0.5mM TCEP, 4ul/25ml Benzonase and 600mg/500ml 4-(2-Aminoethyl)-Benzenesulfonyl Flouride Hydrochloride (AEBSF)  
Vortex and mix vigorously to dislodge the cell pellet  
Sonicate the pellets until all residual biomass is dissolved in resuspension buffer  
Add 10ml Resuspension buffer with 1.5% DDM, 0.5mM TCEP and 600mg/500ml (AEBSF) to each 50ml falcon tube  
Rotate falcon tubes end over end (Roto Shake Genie) at 4°C for 1 hour or until lysate is clear  
Clear lysate by centrifugation, 5000xg for 20 minutes at 4°C

*Nickel affinity purification*

Preparation of 50/50 Ni Slurry:

Pour desired amount of Ni resin into a 50ml Falcon tube  
Centrifuge at 500xg for 2 minutes, pour off supernatant  
Add fresh resuspension buffer to resuspend Ni resin  
Centrifuge again at 500xg for 2 minutes, pour off excess resuspension buffer  
Add equal amount resuspension buffer to pelleted Ni resin and mix

Purification of fusion proteins:

In separate fresh 50ml falcon tubes add 200µl 50/50 Ni slurry (equal parts resuspension buffer and Ni Sepharose Fast Flow)  
Pour centrifuged and cleared lysates into fresh falcon tubes containing the 50/50 Ni slurry  
Rotate falcon tubes end over end for 1 hour at 4°C to allow proteins to bind to the Ni resin  
Centrifuge the falcon tubes at 500xg for 5 minutes at 4°C  
Pour off supernatant, Ni resin will form a loose pellet, be careful not to disturb  
Pipette the pelleted resin into an unsealed 96 well Thompson filter plate, allow to drip dry  
Seal the filter plate with a Thompson 96 well bottom plate seal  
Add one 1ml ATP wash w/ 5mM ATP, 0.1mM TCEP, 0.05%DDM  
Allow to shake at 600 rpm on Glas-Col plate shaker for 30 minutes at 4°C

Remove the seal and allow it to drip dry  
Add 2x 1ml Wash Buffer B w/ 0.1mM TCEP and 0.05% DDM to the filter plate and allow to drip dry  
Reseal filter plate and add 1ml Wash Buffer B to each well, shake at 600 rpm overnight at 4°C

*Elution:*

The next day unseal the filter plate and allow to drip dry  
Add 2x 1ml Wash Buffer B and allow to drip dry  
Place a 96 well round bottom plate underneath the unsealed filter plate and centrifuge at 500xg for 2 minutes to remove all remaining wash buffer  
Reseal filter plate and add 100ul Elution buffer w/0.1mM TCEP and 0.05%DDM.  
Allow to shake at 600 rpm for 30 minutes at 4°C  
To elute place a 96 well round bottom plate underneath the filter plate and centrifuge at 500xg for 2 minutes  
To elute fully, add another 100ul Elution buffer to the filter plate and place a fresh 96 well round bottom plate underneath, shake at 600rpm at 4°C and again centrifuge at 500xg for 2 minutes