



NEW YORK CONSORTIUM ON MEMBRANE PROTEIN STRUCTURE

**Small-scale Protein Purification
January 2010**

Materials:

Reagents:

Buffers:

Resuspension Buffer: 50mM Tris pH 7.8
300mM NaCl
5% glycerol
20mM Imidazole pH 7.8
1mM MgCl₂
0.5mM TCEP
AEBSF
Benzonase

Wash Buffer: 25mM HEPES pH 7.8
500mM NaCl
0.1mM TCEP
5% glycerol
0.05% DDM
75 mM Imidazole pH 7.8

Elution Buffer: 25mM HEPES pH 7.8
200mM NaCl
0.1mM TCEP
5% glycerol
0.05% DDM
500mM Imidazole pH 7.8

Equipment:

Sonicator Robot
Glas-Col shaking platform
96 well, 2mL filter plate (Thompson Instrument Company catalog number 931919)
Vacuum manifold
Centrifuge with plate rotor
Greiner Bio-one 96 well U-shape microplate (Greiner catalog number 650101)

Methods:*Cell lysis and solubilization:*

Place 500µl of resuspension buffer into each well
Program sonicator robot to 2 cycles under the DEEP WELL function
Add 100µl of resuspension buffer containing 12% DDM
Allow lysate to clear for 1 hour using a Glas-Col shaking platform at 4°C

Nickel affinity protein purification:

Preparation of 50/50 Nickel Slurry:

Pour desired amount of Nickel resin into a clean 50mL Falcon tube
Centrifuge at 500xg for 2 minutes and pour off supernatant
Add fresh resuspension buffer to Nickel resin and resuspend
Centrifuge once more at 500xg for 2 minutes and pour off the supernatant
Add equal amount of resuspension buffer to the pelleted Nickel resin and mix

Purification:

Transfer lysate to a bottom sealed 96 well Thomson filter plate
Add 50µl of a 50/50 Ni Sepharose Fast Flow slurry to each well
Place the filter plate on a Glas-Col shaking platform and set it for 600 rpm at 4°C to bind overnight
The following day, unseal filter plate and place it on a vacuum manifold to draw out the lysate
Reseal filter plate and add 1ml of wash buffer to each well
Shake at 600 rpm for 30 minutes on a Glas-Col shaking platform at 4°C
Unseal filter plate and place it on a vacuum manifold to draw out the wash buffer
Repeat wash with an additional 1ml of wash buffer and allow to shake at 600 rpm for 30 min at 4°C
Vacuum wash buffer from filter plate and place a 96 well U-shape microplate underneath
Centrifuge for 2 minutes at 500xg in a plate rotor to remove excess wash buffer and replace bottom seal on filter plate
Apply 35µl of elution buffer into each well and shake at 600 rpm for 30 minutes at 4°C on a Glas-Col shaking platform
Remove bottom seal and place a new 96 well U-shape microplate underneath

Centrifuge for 2 min at 500xg speed in a plate rotor to collect eluates
Analyze eluates by SDS-PAGE and staining with Coomassie Blue to visualize
expressed proteins