**Microscale Extraction and Purification of Integral Membrane Proteins**
**December, 2008**
**Version 1.0**

**Summary:**
This Immobilized Metal Affinity Chromatography (IMAC)-based protein purification protocol is currently being used at the JCIMPT for small-scale preparation of purified proteins starting with biomass of SF9 cells containing over-expressed membrane proteins. Its main use is the early evaluation and characterization of expression, protein properties, and the design of strategies for large-scale production of samples for crystallization and functional studies. The protocol has been tested and extensively used in preparing and screening GPCR samples using 5 mL of biomass.

**Overview of Steps:**

- Cell lysis and production of membrane preparations containing over-expressed proteins
- Extraction and solubilization by detergents
- IMAC purification
- Quality check and characterization of products

**Materials:**

1. **Reagents:**

   Buffers:

   Buffer A: 50mM Hepes, pH 7.5, 500mM NaCl, 10% glycerol

   Stock solutions:

   1M Hepes, pH 7.5 at 4°C
   5M NaCl
   50% glycerol
   10% DDM with 2% CHS (pH >7 for all CHS to go into solution)
   2M Imidazole

2. **Equipment:**

   Dounce homogenizer
   Centrifuge

**Step by step methodology**

*Lysis and Membrane Preparation:*

a. Add 0.3 mL volume of Lysis Buffer (20mM Hepes, pH 7.5, 20mM KCl, 10mM MgCl2)
b. 20+ strokes of dounce homogenization
c. Spin down (18,000 x g, 30 min, 4°C)

d. High Salt Wash 3-5 times with 0.5 mL Buffer A (50mM Hepes, pH 7.5, 500mM NaCl, 10% glycerol)
e. Freeze at -80 °C in 0.5 mL Storage Buffer (50mM Hepes, pH 7.5, 150mM NaCl, 40% glycerol)

*Membrane Extraction and Solubilization:*

f. Thaw out membrane
g. Add 50mM Hepes, pH 7.5, 500mM NaCl to bring glycerol concentration down to 20% or less
h. Add Iodoacetamide to 1mg/mL final concentration (optional)
i. Add ligand, if any.
j. Add DDM to 1% final concentration (0.02% CHS optional)
k. Stir at 4°C for 1 hour
l. Spin down (18,000xg , 30min, 4°C)
m. Transfer supernatant to fresh tube
n. Add 50mM Hepes, pH 7.5, 500mM NaCl, 10% glycerol to bring DDM concentration down to below 0.5% (preferably below 0.2%), and add Imidazole to bring final concentration to 20mM.

*IMAC Purification*

o. Add 20µL TALON resin (pre-equilibrated with 50mM Hepes, pH 7.5, 500mM NaCl, 10% glycerol, 20mM Imidazole
p. Rock at 4°C for 2 hours
q. Spin down at 700xg, 5min, 4°C
r. Wash resin three times with 600uL Buffer A containing 0.05% DDM and 20mM Imidazole
s. Elute with 60uL Buffer A containing 0.05% DDM and 200mM Imidazole.
t. Quality check with analytical SEC and SDS/PAGE or Westerns when expression levels are low

Please send comments, suggestions, and/or questions to Professor Ray Stevens (stevens@scripps.edu)