Hutson Lab Protocol for mitochondrial extract affinity chromatography (author, Dr. Mohammad Islam)

A. Preparation of protein column using activated CH-Sepharose 4B:

1. Suspend 2 gram of powder in 200 ml of cold 1mM HCl for 10 minutes.
2. Filter and wash the swollen resin with cold 1mM HCl.
3. Dissolve 2mg of protein (Protein should be dialyzed over night in 0.1 M sodium bicarbonate (pH 8.0) containing 0.5 M NaC)l.
4. Mix the protein solution with the resin over night in the cold room.
5. Wash the protein bound resin using cold 0.1 M sodium bicarbonate containing 0.5 M NaCl (pH 8.0).
6. Block excess active groups of the resin with 1 M ethanolamine, pH 8.0 for 1 hour.
7. Wash the resin with cold 0.1 M sodium bicarbonate containing 0.5 M NaCl (pH 8.0) to remove the unbound ethanolamine.
8. Wash the resin with 0.05 M Tris-Cl, 0.5M Nacl, pH 8.0.
9. Wash the resin with 0.05 M sodium acetate, 0.5 M NaCl, pH 4.0.
10. Wash the resin with a neutral buffer (PBS buffer).
11. Store the washed gel at PBS buffer in the presence of sodium azide (0.02% at 4^{o}C.

B. Liver Mitochondria Extraction

1. Resuspend liver mitochondria pellets (100 mg protein) in SB buffer without inhibitor.

SB Buffer:

KPi 25mM	2.97875 g
EDTA 0.2mM	29.225 mg
CHAPS 0.75%	3.75 g
KCl 50mM	1.864 g
DTT 1mM	0.077g
pH 7.4 with 1N KOH before add DTT	

2. Add SB buffer to make 25mg/ml concentration solution (calculate from prepared concentration: roughly 4-4.5ml of SB buffer will work.).
3. Mix gently and completely in centrifuge tube (50ml low speed round bottom) (Use smaller size homogenizer pestle).
4. Transfer the mixture into homogenizer and homogenize by hand. Up & down gently several times. Chaps will bread mitochondria membrane then total protein will come out.
5. Take another tube for balance
6. Ultracentrifuge @ 15,000 rpm 4^{o}C for 30 min)
7. Filter the supernatant with 0.2uM syringe filter
8. Wash the protein column with SB buffer until get the base line.
9. Run the supernatant through the column.
10. Wash the column with SB buffer to get the base line.
11. Wash the column with 15ml 0.025 M KCl (Low concentration of salt will clean up the unbound proteins.).
12. Wash the column with SB buffer until you get the base line.
13. Add 10 mM NADH solution in SB buffer and collect BCATm bound protein. When peak starts and reach to top, collect 2.5-3ml of eluted protein then transfer to glass conical bottom tube. Add ice cold acetone upto the top of tube. Seal with parafilm and mix gently then store in -20^{o}C for overnight.
14. Centrifuse the tube at a low speed (2000 rpm)
15. Discard the supernant and resuspend the protein pellets in 10% ice cold TCA solution.
16. Centrifuse the tube at a low speed (2000 rpm)
17. Discard the supernant and resuspend in 1:1 ice cold ether/ethanol solution.
18. Centrifuse the tube at a low speed (2000 rpm)
19. Discard the supernant and dry up in nitrogen.
20. Use the pellets for 1D or 2D electrophoresis.
21. Cut the spots and send to Dr. Mobley for Mass Spec analysis.