

Whole Cell Protein Prep

Whole cell lysis buffer

H ₂ O	3.43ml
1M TrisCl,pH 8.0	50ul
1M NaCl	750ul
0.5 M EDTA	20ul
10% NP40	250ul
10% Sod. Deoxychorate	250ul
40mM Sod.Orthovanadate	250ul
Complete Proteinase inhibitor	half tablet (Roche,Cat# 1 697 498)
Total volume	5 ml

Dissolve half table in H₂O first, then add the rest

10% sodium deoxycholate

100mg/ml H₂O

40mM sodium orthovanadate

7.35mg/ml H₂O (heated briefly to 37 to dissolve)

10% NP40

1ml/9ml H₂O

1. Sonicate 50-100mg tissue in 500 ul of lysis buffer
Branson sonifier, 50% duty, power 4, 15 bursts
2. Leave on ice for 30 minutes
3. Centrifuge lysate at Maximum speed at 4°C for 20min
4. Transfer cleared supernatant (not including top fat layer) to clean tube
5. Measure protein concentration-should make a 1:40 dilution for measuring
6. Aliquot the lysate and store them in -80°C freezer

Western Notes for PGC-1 α antibody

- Load 100ug of whole cell lysate on 7.5% gel to visualize full length protein
- Run gel until 30-40KDa is at the bottom
- Transfer 1 hour on bench with a cold pack at 100V
- Block for 1 hour with 5% milk in TBST
- Use 1:1000 dilution of PGC-GST antibody (affinity purified) in TBST- 1% BSA
- Incubate primary at 4C overnight
- Dilute secondary (Amersham) 1:5000 in 5% milk in TBS-T
- Use ECL reagent from Amersham