

Homogenization Base Buffer:

Prepare and aliquot in 10 ml fractions at -20°C – check to see for any growth or precipitate before using.

300 mM Sucrose

10 mM Tris-Cl pH 8.0

1 mM EDTA

0.5% Fatty acid free BSA

1 M DTT 0.155 g DTT powder in 1.0 ml milliQ water. Freeze (-20°C) in 0.020 ml aliquot

100 mM PMSF 17.4 mg/ml dissolved in ethanol. Prepare 100 ml, store in tight fitting bottle at -20°C (add fresh just prior to use)

20% Sodium Deoxycholate 2 g in 10 ml water. This will take some time to prepare, use a glass bottle, apply gentle heat while using stir bead. Deoxycholate is an ionic detergent to reduce protein interactions and solubilize mitochondria membrane – release CS from mito into homogenate)

Tissue Homogenization

- Prepare Homogenization Buffer: KEEP ICE COLD. Assemble buffer just before use.
 - 10 ml base buffer
 - 10 µl 1M DTT final conc [1.0 mM]
 - 100 µl 100 mM PMSF – add right before use. Slowly pipet with tip immersed in solution. Mix while adding. Half-life is about 30 min.
- Excise 0.5 -1.0 g of tissue. Record the exact mass of tissue
- Mince and homogenize in in 2ml of homogenization buffer per gram of tissue.
- Using a Potter-Elvehjem glass homogenizer pass 5 times (one pass = up and down through sample) Keep ice cold.
- Divide the solution in half. Tube 1: cytosolic fraction; Tube 2: total lysate
 - *Cytosolic Fraction half will be left as is and centrifuged for PEPCK activity*
 - *Total lysate fraction: add deoxycholate and sonicate to second half to solubilize mitochondria for CS*
- Total Lysate Fraction (tube 2)
 - Add 20 µl of 20% deoxycholate per 1.0 ml homogenate, rotate to mix.
 - Sonicate (3-5 x 5-10 second bursts) on ice.
- Leave Cytosolic fraction (tube 1) as is.
- Centrifuge both total lysate fraction and cytosolic fraction at 18,000 x g (max speed on microcentrifuge) for 30 min. at 4°C. Carefully balance each tube with a blank water tube
- Pipet / collect each supernatant and transfer to a new tube.
 - Cytosolic – Divide into 3 similar volumes, label and freeze.
 - Total lysate – Divide into 3 similar volumes, label and freeze.