

PREPARATION OF MEMBRANE PROTEINS FROM HUMAN TISSUE SAMPLES

Tissues should be snap frozen in liquid nitrogen and stored at -80°C till use
Handle tissues on dry ice until sonication

All manipulations from this point on at 4°C

To 20 mg tissue, add 1 ml ice cold SHB+ (or use glass-teflon tissue grinder)
Sonicate (50% duty cycle, output 4, 30 pulses, Branson Sonifier 250) on ice
Spin 20 min at $1000 \times g$ (4000 rpm in microfuge) to pellet nuclei/unbroken cells

Recover post-nuclear supernatant

Spin at $356,000 \times g$ for 30 min to pellet membranes (microsomes)

(Beckman table top ultracentrifuge, TLA100.2 rotor, 100,000 rpm)

Resuspend membrane pellet in TNES+ SHB+ or RIPA (pipet up and down, then draw through Syringe with 26 g needle several times to homogenize membranes, avoid bubbles)

Quantitate protein using Pierce BCA assay, aliquot for gels

Add Laemli sample buffer and freeze at -20°C if SDS-PAGE not run immediately

Typical yields: For 20 mg tissue, resuspend in 100 μl final volume. Concentrations roughly 3-4 $\mu\text{g}/\mu\text{l}$. Run 50 μg per lane of gel.

For nuclear pellet – take original pellet and resuspend in TNET+

Spin 20 min at $1000 \times g$ (4000 rpm in microfuge) to pellet nuclei

Resuspend in RIPA + complete shear DNA by drawing through syringes $\sim 18\text{g}$

SHB (sucrose homogenization buffer)

0.255 M sucrose

1 mM EDTA

20 mM Tris pH 7.4

SHB+

1X SHB

1 mM PMSF

1 $\mu\text{g}/\text{ml}$ pepstatin A – can substitute 1 X protease complete (Boehringer) for these 2

1 $\mu\text{g}/\text{ml}$ leupeptin - can substitute 1 X protease complete (Boehringer) for these 2

TNES

1 % SDS

150 mM NaCl

50 mM Tris pH 7.4

2 mM EDTA

TNES+

1X TNES

1 mM PMSF

1 $\mu\text{g}/\text{ml}$ pepstatinA

1 $\mu\text{g}/\text{ml}$ leupeptin

TNET

1% Triton X100

150 mM NaCl

50 mM Tris pH 7.4

2 mM EDTA

RIPA

60 μ l NP40

150 μ l 20% SDS

6 μ l PMSF 17 mg/ml MCOH

240 μ l 25x complete

6 mls