Soluble (S-100) mitochondrial fractionation protocol for western blot

Materials

Mitochondrial lysis buffer

25 mM HEPES KOH, pH 7.6
5 mM $MgCl_2$
0.5 mM EDTA
10% glycerol
1 mM DTT
1 mM PMSF

Procedure

1. Resuspend mitochondria in one-third of the packed cell volume with mitochondrial lysis buffer. Put the suspension into a glass homogenizer and homogenize with 10 strokes using a tight pestle. Add Tween 20 and KCl to final concentrations of 0.5% and 0.5 M, respectively.
2. Incubate the mixture on ice for 5 min. Repeat the homogenization 10 times.
3. Spin the final mitochondrial lysate at 100,000 g in an ultracentrifuge using a TY65 Beckman rotor at 4°C, for 1 h.
4. Carefully collect the clear supernatant, avoiding the fluffy layer over the pellet, to yield the final S-100 fraction.
5. Freeze in aliquots, in liquid nitrogen, and store at -80°C.

This is a slightly modified protocol taken from: Micol V, Fernandez-Silva P and Attardi G (1996). Mitochondrial Biogenesis and Genetics Part B. *Methods of Enzymology*, Volume 264.

For convenience please consider our Mitochondria Isolation Kit for Cultured Cells (ab110171).