Mitochondrial purification protocol for western blot samples

Materials

NKM buffer

1 mM Tris HCl, pH 7.4
0.13 M NaCl
5 mM KCl
7.5 mM $MgCl_2$

Homogenization buffer

10 mM Tris-HCl
10 mM KCl
0.15 mM $MgCl_2$
1 mM PMSF
1 mM DTT

Always add PMSF and DTT immediately before use.

Mitochondrial suspension buffer

10 mM Tris HCl, pH 6.7
0.15 mM $MgCl_2$
0.25 mM sucrose
1 mM PMSF
1 mM DTT

Procedure

1. Collect cells by centrifugation at approximately 370 g for 10 min. Decant supernatant and resuspend cells in 10 packed cell volumes of NKM buffer.
2. Pellet cells and decant supernatant, repeat this washing step 2 times. Resuspend cells in 6 packed cell volumes of homogenization buffer.
3. Transfer cells to a glass homogenizer and incubate for 10 min on ice. Using a tight pestle homogenize the cells. Check under the microscope for cell breakage, the optimum is around 60%. This may require 30 strokes or so of the pestle.
4. Pour homogenate into a conical centrifuge tube containing 1 packed cell volume of 2 M sucrose solution and mix gently. Pellet unbroken cells, nuclei and large debris at 1,200 g for 5 min and transfer the supernatant to another tube. This treatment is repeated twice, transferring the supernatant to a new tube each time, discarding the pellet.
5. Pellet the mitochondria by centrifuging at 7,000 g for 10 min. Resuspend the mitochondrial pellet in 3 packed cell volumes of mitochondrial suspension buffer. Spin at 9,500 g for 5 min to re-pellet the mitochondria.
6. At this point, you can add 1X protein gel loading buffer and run on a gel if a whole mitochondrial protein extract is needed, further purify the mitochondria on a sucrose gradient if you really need very pure mitochondria, or purify a soluble (S-100) fraction of the mitochondria. See our soluble (S-100) mitochondrial fractionation protocol.

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