



**Isolation of Mitochondrial fraction (This protocol is for use with non-adherent cells)**

1. Collect cells by centrifugation at approximately 370 x g for 10 minutes. Decant supernatant and re-suspend cells in 10 packed cell volumes of NKM buffer (1 mM Tris-HCl, pH 7.4, 0.13 M NaCl, 5 mM KCl, 7.5 mM MgCl<sub>2</sub>).
2. Pellet cells and decant supernatant, repeat this washing step 2 times. Resuspend cells in 6 packed cell volumes of homogenization buffer (10 mM Tris-HCl, pH 6.7, 10 mM KCl, 0.15 mM MgCl<sub>2</sub>, 1 mM PMSF, and 1 mM DTT, always add PMSF and DTT immediately before use).
3. Transfer cells to a Dounce homogenizer and incubate for 10 minutes 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 mixed gently. Pellet unbroken cells, nuclei, and large debris at 1200 g for 5 minutes 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 7000 g for 10 minutes. Resuspend the mitochondrial pellet in 3 packed cell volumes of mitochondrial suspension buffer (10 mM Tris-HCl, pH 6.7, 0.15 mM MgCl<sub>2</sub>, 0.25 mM sucrose, 1 mM PMSF, 1 mM DTT). Spin at 9500 g for 5 minutes 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.