

Cell lyses and Fractionation: Nuclear and Cytosolic fractions

1. Harvest cells and wash two times with ice cold PBS, centrifuge at 1200rpm for 7 min at 4C
2. Resuspend the pellet in 0.33 M sucrose, 10 mM Hepes pH 7.4, 1mM MgCl₂, 0.1% Triton X-100 in 5:1 v/v (buffer should be ice cold and protease inhibitors can be added)
3. Leave on ice for 15 minutes, try to break up aggregates gently with thin glass rod
4. Spin at 3000rpm in microfuges for 5 min at 4°C. Collect the supernatant, label as cytosolic and store at -70°C
5. Repeat steps 2 thru 4 but discard this supernatant. The pellet should contain a pure preparation of nuclei without the nuclear membrane.
6. To the nuclear pellet add 0.45M NaCl, 10 mM Hepes pH 7.4 (ice cold and you may add protease inhibitors) and resuspend gently on ice.
7. Incubate the suspension on ice for 15 min and at 5 min intervals, flick to resuspend. Under these conditions most nuclear proteins will be extracted, do not exceed 0.5M NaCl or you will extract histone.
8. Spin at maxi speed in a microfuges for 5 min at 4°C
9. Collect the supernatant and store in aliquots, this is your nuclear extract.