

Preparation of Nuclear Extract from Plated Cells

Solution NE1:

10 mM Hepes pH 8.0
1.5 mM MgCl₂
10 mM KCl
1 mM DTT

Solution NE2:

20 mM Hepes pH 8.0
1.5 mM MgCl₂
25% Glycerol
420 mM NaCl
0.2 mM EDTA
1 mM DTT
0.5 mM PMSF

**Cells should be 80% confluent for extraction. 10 x 10 cm dishes of cells yields around 50 ul of nuclear extract.

1. Take up cells in 1 ml PBS per plate. Place in eppendorf and spin at 1K for 2 min.
2. Resuspend all the pellets together in 1 ml PBS. Spin at 1K for 2 min.
3. Repeat step 2.
4. Take off last of PBS and estimate packed cell volume (PCV). Resuspend in 1 PCV of NE1. Leave on ice for 15 mins.
5. Take up suspension into a 1 ml syringe that has been prewashed with buffer NE1.
6. In one stroke, force the cells through a 23 gauge needles into an eppendorf. Take back into syringe and repeat a further 4 times.

7. Microfuge at full speed for 20 sec.
8. Discard supernatant and resuspend nuclear pellet in 2/3 PCV of NE2.
9. Leave on ice with regular stirring for 30 min.
10. Pellet nuclear debris for 5 min at full speed.
11. Dialyze supernatant against standard nuclear extract buffer for 2 hours.