# Preparation of Nuclear Extract from Plated Cells

## Buffers

| Solution NE1 | |
|---|---|
| **HEPES pH 8.0** | 10mM |
| **$MgCl_2$** | 1.5mM |
| **KCL** | 10mM |
| **DTT** | 1mM |

| Solution NE2 | |
|---|---|
| **HEPES pH 8.0** | 20mM |
| **$MgCl_2$** | 1.5mM |
| **Glycerol** | 25% |
| **NaCl** | 420mM |
| **EDTA** | 0.2mM |
| **DTT** | 1mM |
| **PMSF** | 0.5mM |

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

## Procedure

1. Take up cells in 1ml PBS per plate. Place in eppendorf and spin at 1000rpm for 2 minutes.
2. Re-suspend all the pellets together in 1ml of PBS. Spin at 1000rpm for 2 minutes.
3. Repeat step 2.
4. Take off last of PBS and estimate packed cell volume (PCV). Re-suspend in 1 PCV of NE1.
5. Leave on ice for 15 minutes.
6. Take up suspension into a 1ml syringe that has been pre-washed with buffer NE1.
7. In one stroke, force the cells through a 23 gauge needle into an eppendorf. Take back into syringe and repeat 4 times.
8. Microfuge at full speed for 20 seconds.
9. Discard supernatant and re-suspend nuclear pellet in 2/3 PCV of NE2.
10. Leave on ice with regular stirring for 30 minutes.
11. Pellet nuclear debris for 5 minutes at full speed.
12. Dialyze supernatant against standard nuclear extract buffer for 2 hours.