Nuclear Fractionation Protocol

Reagents

Buffer A
10 mM HEPES, 1.5 mM MgCl2, 10 mM KCl, 0.5 mM DTT,
0.05% NP40 (or 0.05% Igepal or Tergitol) pH 7.9

To prepare 250 ml stock of buffer A –
HEPES: 1M = 238.3 g/L, therefore 10 mM = 0.59 g/250ml
MgCl2: 1M = 203.3 g/L, therefore 1.5 mM = 0.076 g/250ml
KCl: 1M = 74.5 g/L, therefore 10 mM = 0.187 g/250ml
DTT: 1M = 154.2 g/L, therefore 0.5 mM = 0.019 g/250ml
NP40 = 0.05%

Buffer B
5 mM HEPES, 1.5 mM MgCl2, 0.2 mM EDTA, 0.5 mM DTT,
26% glycerol (v/v), pH 7.9

To prepare 250 ml stock of buffer B –
HEPES: 1M = 238.3 g/L, therefore 5mM = 0.295 g/250ml
MgCl2: 1M = 203.3 g/L, therefore 1.5mM = 0.076 g/250ml
EDTA: 1M = 372.2 g/L, therefore 0.2mM = 0.0186 g/250ml
DTT: 1M = 154.2 g/L, therefore 0.5mM = 0.019 g/250ml
26% Glycerol (v/v) = 65ml
4.6 M NaCl – 87.66 g/326ml

Method

1. Prepare 1 ml of buffer A with added cocktail of usual inhibitors from frozen stock and store on ice.
2. Add 500 µl of buffer a per large petri dish on ice and scrape thoroughly, leave on ice for 10 min.
3. Centrifuge at 4°C at 3000 rpm for 10 min.
4. Remove supernatant and keep it (this will contain everything except large plasma membrane pieces, DNA, nucleoli), extract out 10 µl for Bradford assay.
5. On ice resuspend pellet in 374 µl of buffer B and add 26 µl of 4.6 M NaCl to give 300mM NaCl (high salt helps lyse membranes and forces DNA into solution).
6. Homogenize with 20 full strokes in Dounce or glass homogenizer on ice.
7. Leave on ice for 30 min.
8. Centrifuge at 24,000 g for 20 min at 4°C.
9. Aliquot supernatant, remove 10 µl for Bradford assay and store at -70°C.