

Crude Nuclear Extracts

All following steps should be performed at 0°C.

Step 1:

Tissue samples:

Wash tissue sample (0.2-0.3 cm³) once in 2 ml buffer A.
Aspirate buffer A and add another 2 ml buffer A
Homogenize tissue, using a Polytron homogenizer.
Spin down cells, 10' at 1,700 g.

Monolayer cell cultures (10 cm plate):

Harvest cells with 2 ml TEN, and spin down 10' at 1,700 g.
Wash once in 2 ml PBS and spin down 10' at 1,700 g.
Wash once in 2 ml buffer A and spin down 10' at 1,700 g.

Step 2:

Re-suspend pellet from **Step 1** in 1 ml buffer NE.
Extract cells 30'
Spin down cellular debris 20' at 20,000 g.
transfer supernatant to new tube.
Store at -80°C.

Solutions:

TEN:	40 mM Tris-Cl pH 7,5 1 mM EDTA 150 mM NaCl
Buffer A:	25 mM Tris pH 7,5 50 mM KCl 2 mM MgCl ₂ 1 mM EDTA 5 mM dithiothreitol (DTT)
Buffer NE:	25 mM Tris pH 7,5 0.42 M NaCl 1.5 mM MgCl ₂ 0.5 mM EDTA 1 mM dithiothreitol (DTT) 25% Sucrose