Nuclear Extract Protocol (F. Cogent)

- Cells washed with PBS and resuspended in
  Buffer A: HEPES 20mM pH7.8
  EDTA 0.15mM
  EGTA 0.15mM
  KCl 10mM

- NP40 added to a final concentration of 0.25% and the cell incubated on ice for 20-30 mins.

- Spin out membranes and cellular debris containing cytoplasmic fraction and a small amount of nuclear proteins at 3000rpm 5min at 4°C

- The pelleted nuclei washed in Buffer B: HEPES 20mM pH7.8
  NaCl 140mM
  Glycerol 25%
  EDTA 0.1mM

- Resuspend in Buffer C: HEPES 20mM pH7.8
  NaCl 400mM
  Glycerol 25%
  EDTA 0.1mM

- Incubate on ice for 30 mins and the nuclei was spun out 20 mins at 10,000g and the nuclear extract recovered and stored frozen at -80°C

† PMSF may be added at final conc. 1mM
Dear Dr Robertson;
In response to your mail, please find enclosed the condition of our experiments.

Nuclear extracts:
293T cells were transfected, washed with PBS and resuspended in buffer A (Hepes 20mM EDTA 0,15mM, EGTA 0,15mM, KCL 10mM) and lysed with NP40 (0,25% final) The nuclei were washed with buffer B (Hepes 20mM pH 7,8, NaCl 0,1M, glycerol 25% and EDTA 0,1mM) and resuspended in buffer C (buffer B with NaCl 0,4 M) with NaCl added to a final concentration of 0,4M. After 30 min at 4 C, the nuclei were spun out (20 min at 10000g) and the nuclear extract recovered.

Gel shift assays
Nuclear extracts (5 microg) were incubated with antibody or not in binding buffer at 4 C for 1 h.
Binding buffer: Hepes 20mM ph 7,5 KCL 70mM, DTT 2mM, NP40 0,01%, Ficoll 4%, BSA 100 microg/ml, MgCl2 10mM
and then with labelled oligonucleotide for 20 min. at room temperature and loaded onto a 4% polyacrylamide gel in 0,5 TRIS borate EDTA buffer.

Best Regards
F. Logeat (A. Israel)