Nuclear Extract Protocol (F. Logeat)

- Cells washed with PBS and resuspended in

Buffer A : HEPES 20 mM pH 7.8
EDTA 0.15 mM
EGTA 0.15 mM
KCl 10 mM

- NP40 added to a final concentration of 0.25% and the cell incubated on ice for 20-25 mins.
- Spin out Membranes and cellular debris containing cytoplasmic fraction and a small amount of nuclear proteins at 3000 rpm 5 mins at 4°C
- The pelleted Nuclei washed in Buffer B: Hepes 20 mM pH 7.8
NaCl 100 mM
glycerol 25%
EDTA 0.1 mM

- Resuspend in Buffer C : Hepes 20 mM pH 7.8
NaCl 400 mM
glycerol 25%
EDTA 0.1 mM

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

* PMSF may be added at final conc. 1 mM

Date: Tue, 7 Nov 1995 10:34:13 +0100 (MET)
From: Frederique Logeat <flogeat@pasteur.fr>
To: esrobert@bustoff.bwh.harvard.edu
Subject: Notch

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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% plyacrilamide gel in 0,5 TRIS borate EDTA buffer.
Best Regards
F. Logeat (A. Israel)