Nuclear Protein Extract — 6/30/95

Buffer A

10 mM HEPES pH 7.9	– 100 µl	1 M
10 mM KCl	– 100 µl	1 M
1.5 mM $MgCl_2$	– 15 µl	1 M
dH_2O	– to 10 mls	
	10 mls	

Buffer B

20 mM HEPES pH 7.9	– 200 µl	1 M
10% Glycerol	– 1000 µl	100%
420 mM NaCl	– 840 µl	5 M
1.5 mM $MgCl_2$	– 15 µl	1 M
0.2 mM EDTA	– 4 µl	0.5 M
dH_2O	– to 10 mls	
	10 mls	

Add
- 1 M DTT – 50 µl / 10 ml
- 100 mM PMSF – 50 µl / 10 ml
- 10 mg/ml Aprotinin – 10 µl / 10 ml

- add just before use to both A and B buffers on ice
- Collect cells ~ 50 → 100 x 10⁶ at 1–2000 rpms 10 mins 4°C
- Wash cells once in 10 – 25 mls PBS. Pellet as above
- resuspend in 4 Vols. to size of pellet Buffer A ≈ 500 – 100 µl.
- Incubate on ice for 1 hour.
- Transfer to dounce and homogenize 20 – 30 Strokes
- transfer to 1.5 ml epp. tube. Centrifuge @ 2000 rpms 5 mins 4°C
- Aspirate and resuspend in 1 ml Buffer A.
- Centrifuge @ 2000 rpms 5 mins, 4°C. Aspirate.
- Resuspend in 3 Vols. pellet with buffer B.
- incubate on ice 30 mins.
- Microfuge 20 mins @ 13,000 rpms 4°C.
- Transfer to fresh 1.5 ml epp.
- Snap freeze. –80°C

Dilution 1:200

Bradford Assay:
- 795 µl H_2O
- 5 µl protein (NE)
- 200 µl Bradford
- 1000 µl

Buffer A Extraction Buffer

		10 mls	
100 mM	Tris-Cl pH 9.0	1 ml	1 M Tris-Cl pH 9.0
100 mM	NaCl	1 ml	1 M NaCl
5 mM	KCl	50 µl	1 M KCl
0.5 mM	$MgCl_2$	5 µl	1 M $MgCl_2$
1 mM	$CaCl_2$	10 µl	1 M $CaCl_2$
0.5%	NP40	500 µl	10% NP40

Increase to 10 mls with dd H_2O

Buffer B Gradient Buffer

		50 mls	
10 mM	HEPES pH 7.8	500 µl	1 M HEPES pH 7.8
5 mM	Na_2HPO_4	250 µl	1 M Na_2HPO_4
5 mM	KCl	250 µl	1 M KCl
0.5 mM	$MgCl_2$	25 µl	1 M $MgCl_2$
1 mM	DTT	500 µl	100 mM DTT.
100%	NP40	(250 µl	0.5% NP40 final conc)