PREPARATION OF NUCLEAR EXTRACTS
FOR IN VITRO TRANSCRIPTIONS, FOOTPRINTING, ETC.

All buffers, autoclave then add HEPES or filter sterilize

Nuclei extract buffers: *Dignam et al., Nuc. Acid Res. 11, 1475-1489*

A:	10 mM HEPES (pH7.9 at 4°C)	1 ml	1 M
	1.5 mM $MgCl_2$	150 µl	1 M
	10 mM KCl	500 µl	2 M
	[Add fresh 0.5mM DTT]		
		TVf = 100mls	
B:	0.3 M HEPES 7.9	30 mls	1 M
	1.4 M KCL	70 mls	2 M
	0.03 M MgCl2	3 mls	1 M
		TVf = 100 mls	
C:	20 mM HEPES 7.9	2 mls	1M
	25% v/v glycerol	50 mls	50%
	0.42 M NaCl	10.5 mls.	4M
	1.5 mM $MgCl_2$	150 µl	1M
	0.2 mM EDTA	40 µl	0.5 M
	[Add PMSF to 0.5mM fresh]		
	[0.5 mM DTT fresh]		
		TVf = 100mls	
D:	20 mM HEPES 7.9	2 mls	1 M
	20% v/v glycerol	40 mls	50%
	0.1 M KCL	5 mls	2 M
	0.2 mM EDTA	40 µl	0.5 M
	[0.5 mM DTT]		
	[Add DMSF to 0.5 mM fresh]		
		TVf = 100 mls	

Stocks: 100x DTT = 50 mM or 200x = 100 mM = 15.4 mg/1 ml
100x PMSF = 50 mM 200x = 100 mM = 77 mg/ml
1M HEPES 7.9 = 23.8 g/100mls 0.01 M = .238 g/100mls
4 M KCL = 29.82 g/100mls
50% glycerol need 100mls
1 M $MgCl_2$ = 20.33 g/100mls
PBS

(FOR HELA CELLS)

TEN MAXI PLATES--~2-2.5 x 10^8 HeLa cells
Pellet ~2.5mls

Buffer: A - 20 mls
C - 1 ml
D - 200 mls
B - 1 ml

1. Wash cells w/PBS, ice cold.
2. Scrape in 5 mls PBS/Plate.
3. Pellet at 2000 RPM 10' clinical centrifuge.
4. Resuspend in 5 x vol. Buffer A, 4°C, ice 10' ~12mls.
5. Pellet at 2000 RPM 10', 4°C.
6. Resuspend in 2 x vol. Buffer A ~5mls.
7. Homogenize ~20 strokes.
8. Check lysis on microscope.
9. Pellet at 2000 RPM 10',4°C.

From this point, treat the pellet and supernatant separately.

A. Pellet = nuclei
1. Transfer to ultracentrifuge tubes.
2. Pellet at 25,000g 4°C = 17,000 RPM.
3. Resuspend in 0.6 mls buffer C.
4. Homogenize, Vf~1.2-1.5 ml.
5. Stir w/magnet 30' at 4°C.
6. Pellet at 25,000g, 30'.
7. Dialysis SUPER (~1ml) against buffer D.
for 5 hours
8. Pellet at 25,000g, 20'
9. SUPER = nuclear extract
freeze

expect 6-8 mgs/ml

B. Super = cytoplasmic extract
1. Add to Super 0.11 vol. buffer B (e.g., 6 mls + 0.66 ml buffer B)
2. Spin 100,000 g, 1 hr. at 4°C.
3. Dialysis of Supernatent (~4-5 mls) against buffer D (200 mls) for 5-8 hours.
4. This is S100 fraction. Freeze