PREPARATION OF NUCLEAR EXTRACTS & S100 SUPERNATANT FRACTIONS *FOR IN VITRO TRANSCRIPTIONS, FOOTPRINTING, ENZYME ASSAYS 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 MgCl2	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
1.5mM $MgCl_2$	150µl	1M
0.5mM ATP (optional)	0.5 ml	0.1M
[0.5 mM DTT]		
[Add PMSF 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

0.1M ATP 60mg/0.8mL; adjust with NaOH to pH 7.0

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' (12 mls).
5. Pellet at 2000 RPM 10', 4°C.
6. Resuspend in 2 x vol. Buffer A (5mls.)
7. Homogenize 20 strokes with dounce homogenizer.
8. Check lysis on microscope, should be complete, if not do more, keep on cold though.
9. Pellet at 2000 RPM 10',4°C.

From this point, treat the pellet and supernatant separately; one can create either nuclear or supernatant extracts.

A: Pellet, Pellet = nuclei= nuclear extracts
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, Vf1.2-1.5 ml.
5. Stir w/magnet 30' at 4°C.
6. Pellet at 25,000g, 30'.
7. Dialysis this supernatant (1ml) against buffer D.for 5 hours
8. Pellet at 25,000g, 20'
9. Supernatant = nuclear extract, freeze –70C, expect 6-8 mgs/ml; aliquot for best results.

B.
Supernatant = 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. Use ATP for proteosome and other energy dependent systems if used directly or add later for best activity.
4. This is **S100** fraction, aliquot and freeze at –70C, use this for enzyme assays etc.