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 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 ml 2 M
    0.03 M MgCl₂ 3 ml 1 M
    TVf = 100 mls

C: 20 mM HEPES 7.9 2 ml 1 M
    25% v/v glycerol 50 ml 50%
    0.42 M NaCl 10.5 ml 4 M
    1.5 mM MgCl₂ 150 µl 1 M
    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 ml 1 M
    20% v/v glycerol 40 ml 50%
    0.1 M KCl 5 ml 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 MgCl₂ = 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.
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 = cytoplasmonic 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