

Cellular Nuclear Extracts for DNase Footprinting.  
( from Dignam et. al. Methods in Enzymology, Vol. 101)

1) Harvests cells from tissue culture media by centrifugation at 800-1000 RPM for 15 min. (in Sorval in 500 ml bottles if doing a large scale prep.)

Preform next steps at 0°-4° .

2) Wash 2x with cold PBS. ( 1x 100ml, 1x 50ml in 50ml[Falcon blue cap.] )

3) Determine packed vol. of cells by centrifugation for 10 min. at 800-1000 RPM.

4) Resuspend cells in 5 vols. (from #3) of buffer A and allowed to stand on ice for 10 minutes. Collect cells by centrifugation.

5) Resuspend cells in 2 vols. of buffer A. Homogenize cells by 10-12 strokes of a glass Dounce homogenizer (B pestle).

6) Check cells under microscope for lysis (greater than 90%) if not properly lysed try a few (4-5) additional strokes. (Transfer to tubes for the Beckman JA 20.1 rotor)

7) Spin homogenate for 10 min at 1000 RPM. Carefully collect supernatant. Spin again for 20 min at 25,000 g. Collect supernatant and combine with first spin supernatant, (save the nuclear pellet), add 0.11 vol of buffer B. Centrifuge for 60 min at 100,000 g. The resulting supernatant is dialyzed against 20 vols. of buffer D for 4-5 hours.

This is called the S100 Fraction. (For footprinting S100 fraction does not usually show activity) .

8) Homogenize nuclear pellet from above in 2.5ml per  $10^9$  cells of buffer C applying 10 strokes of a Dounce homogenizer (B pestle). Stir homogenate on ice for 30 mins.

9) Centrifuge this mixture for 30 mins. at 25,000 g. Dialyze for 4-5 hrs. against 50 vols. of buffer D.

This called the nuclear extract.

10) Centrifuge both the S100 and nuclear extract at 25,000 g for 30 mins.

11) These are stored by quick freezing in liquid nitrogen and stored at -70°. These are stable for at least 4 months.

Buffers for making nuclear extracts.

**Buffer A**

Final	Stock	100 ml
10mM HEPES (pH 7.9)	1M	1 ml
1.5mM Mg Cl <sub>2</sub>	1M	150 ul
10 mM K Cl	1M	1 ml
0.5mM DTT	1M	50 ul
0.5 mM PMSF	.25M	20 ul

**Buffer B**

0.3M HEPES (pH7.9)	1M	30 ml
30 mM Mg Cl <sub>2</sub>	1M	3 ml
1.4 M K Cl		10.43 gm

**Buffer C**

20mM HEPES (PH7.9)	1M	2 ml
25% glycerol	100%	25 ml
0.42M Na Cl	1M	42 ml
1.5mM Mg Cl <sub>2</sub>	1M	150ul
0.2mM EDTA	.5M	10 ul
0.5mM PMSF	.25M	20ul
0.5mM DTT	1M	50ul

**Buffer D**

			4 liters
20 mM HEPES (pH7.9)	1M	2 ml	80 ml
20% glycerol	100%	20 ml	800 ml
0.1M K Cl	1M	10 ml	400 ml or 29.80 gm solid
0.2mM EDTA	0.5M	10 ul	400 ul
0.5mM DTT	1M	50 ul	2 ml
0.5mM PMSF	.25M	20 ul	800ul

Note; Add DTT and PMSF just before use. Dissolve PMSF in ethanol or isopropanol for a 0.25M stock solution. Add PMSF to solutions dropwise and stir so that it will go in to solution.