

Chromatin Isolation From REF52 Cells

1. Plate 2×10^5 cells, grow overnight (3 dishes)
2. Scrape 4×10^5 cells, spin down at 5K 4°C
3. Lyse in 1 ml CSK buffer
4. Incubate on ice, 20 mins
5. Centrifuge at 300g at 4°C for 5 min
6. Wash pellet with 1 ml lysis buffer for 5 mins on ice
7. For nuclease treatment, resuspend in lysis buffer with 1 mM CaCl_2 and add nuclease.
8. Centrifuge again.
9. Quantitate by BCA.

CSK Buffer

10 mM Pipes, pH 7	10 ml from 0.05 M Stock
100 mM NaCl	1 ml from 5M Stock
300 mM Sucrose	15 ml from 1M Stock
3 mM MgCl_2	150 ul from 1M Stock
0.5% Triton X-100	250 ul
1 mM orthovanadate	9.2 mg
25 mM B glycerolphosphate	270.0 mg
H ₂ O	22 ml

Alliquot out 5 ml

Supplement with

1mM ATP	50 ul of 100 mM
0.1 mM Benzensulfony fluoride (10 mM stock= 10 mg/4.2 ml) mM	50 ul of 10 mM
2 ug/ml pepstatin	10 ul of 1 mg/ml
10 ug/ml leupeptin	50 ul of 1 mg/ml
5ug/ml phostivin	5 ul of 1 mg/ml

From Sillman

1. 3×10^6 cell washed with PBS, resuspend in 200 ul solution A (4×10^7 /ml)
2. Add Triton-X to a final concentration of 0.1% (20 ul of 1%)
3. Ice for 5 mins
4. Cytoplasmic proteins (S1) separated from nuclei (P1) by 1300g for 4 mins.
5. Further clarify S1 by spinning high speed for 15 mins, save supernatant.
6. Add 500 ul to P1
7. Wash

8. Resuspend in 200 ul
9. Divide nuclei in half (100 ul each).
10. Digest with nuclease
11. Lyse nuclei with 100-200 ul solution B
12. Incubate on ice for 30 mins
13. Separate soluble nuclear proteins (S2) from chromatin (P2) by centrifugation 1700g for 4 mins
14. Wash chromatin with solution B
15. Spin 1700 for 4 mins
16. Resuspend chromatin in 100 ul SDS sample buffer and shear with sonication

To digest chromatin:

1. Incubate P1 in 100 ul solution A containing 1 mM CaCl₂ (1 ml buffer A + 1 ul 1M CaCl₂) and 1 units of micrococcal nuclease (5 ul) 37 degree for 30 min.
2. Add 1 mM EGTA.
3. Spin 1300g X 4 mins
4. Lyse with Solution B for 30 mins on ice
5. Spin 1700 g for 4 mins
6. Resuspend in 100 ul SDS

Quantitate with BCA

Solution A

10 mM Hepes pH 7.9	1M	0.5 ml
10 mM KCl	1M	0.5 ml
1.5 mM MgCl ₂	1M	75 ul
0.34 M sucrose	1M	17 ml
10% glycerol		5 ml
10 mM DTT		77 mg
10 mM NaF		21 mg
1 mM Na ₂ VO ₃		9.2 mg
Protease inhibitors		
H ₂ O		27 ml

Solution B

3 mM EDTA	0.5 M	60 ul
0.2 mM EGTA	0.1M	20 ul
1 mM DTT	0.5 M (77 mg/ml)	20 ul
H ₂ O		10 ml
Protease inhibitor		