Chromatin Isolation by small-scale biochemical Fractionation


Buffer A
10 mM HEPES, pH 7.9
10 mM KCl
1.5 mM MgCl2
0.34 M Sucrose
10 % Glycerol
1 mM DTT
Protease inhibitor cocktail

Buffer B
3 mM EDTA
0.2 mM EGTA
1 mM DTT
Protease inhibitor cocktail

1. Harvest 1 x 10^7 – 2 x 10^7 cells by using a cell scraper; spin down at 1000 rpm for 2 min, discard supernatant
2. Wash cell pellet with PBS, spin down at 1000 rpm for 2 min.
3. Repeat second step.
4. Resuspend cell pellet in 200 µl of Buffer A.
5. Add Triton X-100 to a final concentration of 0.1%.
6. Incubate cells on ice for 8 min.
7. Centrifuge at 1,300 x g, 4 °C, for 5 min; separate supernatant = fraction S1 from pellet (nuclei) = fraction P1.
8. Clarify S1 by high-speed centrifugation at 20,000 x g, 4 °C, for 5 min; collect supernatant= fraction S2 (discard P2)
9. Wash P1 once with Buffer A and lyse it for 30 min in Buffer B (100 µl).
10. Centrifuge at 1,700 x g, 4 °C, for 5 min; separate supernatant = fraction S3 from pellet (chromatin) = fraction P3.
11. Wash P3 once with Buffer B and resuspend it either in SDS sample buffer (then boil for 10 min at 70 °C and analyse chromatin associated proteins by SDS PAGE/Western Blot) or in nuclease digestion buffer.