Extraction of Crude Nuclear Extract for HDAC assay

Buffers

*Lysis Buffer
10 mM Tris HCl pH7.5
10 mM NaCl
15 mM $MgCl_2$
250 mM Sucrose
0.5 % NP-40
0.1 mM EGTA

*Sucrose cushion
30 % Sucrose
10 mM Tris HCl pH7.5
10 mM NaCl
3 mM $MgCl_2$

*Extraction buffer
50 mM Hepes KOH, pH 7.5, 420 mM NaCl, 0.5 mM EDTA Na_2, 0.1 mM EGTA, 10 % glycerol.

Procedure

Isolation of Nuclei

1. Suspend 1x 10^7 cells (100 mm dish sub-confluent) into 1ml of lysis buffer.
2. Vortex for 10 second.
3. Keep on ice for 15 min.
4. Spin the cells through 4 ml of sucrose cushion at 1,300 x g for 10 min at 4 C.
5. Discard the supernatant.
6. Wash the nuclei pellet once with cold 10 mM Tris HCl pH7.5, 10 mM NaCl.

Extraction of Nuclei

1. Suspend the isolated nuclei in 50-100 ul of extraction buffer.
2. Sonicate for 30 seconds.
3. Stand on ice for 30 min.
4. c.f.g. 15,000 rpm for 10 min.
5. Take supernatant (the crude nuclear extract).
6. Determine protein conc. by Bradford method or equivalent.
7. Store the crude nuclear extract at –70 C until use.

Note: Do not use any kind of protease inhibitor!!