Nuclear Isolation for Run-on Assay or for Nuclear Extracts

7-16-99 TJM

1. In case of RASM, 5 of 10 cm or 2-3 of 15 cm dishes are necessary for one experiment.
2. Wash once with PBS (RT).
3. Collect cells with 5 ml of ice cold PBS. Spin in a Beckman GS-6 rotor at 1,500 rpm, 4C for 5 min.
4. Resuspend the cells with 10 ml of solution1. Spin in a Beckman GS-6 rotor at 1,500 rpm, 4C for 5 min.
5. Resuspend the pellet with 2 ml of solution 2. Incubate on ice for 5 min.
6. Transfer the cells to a glass Dounce homogenizer and homogenize with10 strokes using pestle B.
7. Layer the cells over 4 ml of solution 3 and spin in a Beckman GS-6 rotor at 2,000 rpm, 4C for 10 min.
8. If doing a run-on assay, resuspend the nuclei with solution 4 to 200 ul and freeze in liquid N_2. If using the nuclei to make protein extracts, proceed with nuclear pellet with the extraction protocol.

Solution 1

	stock	for 500 ml
10 mM Tris/HCl, pH 7.4	1 M	5 ml
150 mM KCl	1 M	50 ml
4 mM magnesium acetate	40 mM	50 ml
qH_2O		395 ml

Solution 2

	stock	for 100 ml
10 mM Tris/HCl, pH 7.4	1 M	1 ml
150 mM KCl	1 M	10 ml
4 mM magnesium acetate	40 mM	10 ml
0.5 % Nonidet p-40		0.5 ml
qH_2O		78.5 ml

Solution 3 (1.8 M sucrose cushion)

	stock	for 20 ml
100 mM Tris/HCl, pH 7.4	1 M	2.0 ml
5 mM $MgCl_2$	1 M	0.1 ml
sucrose		12.3 g

Add qH_2O, dissolve and make up volume to 20 ml. Filter sterilize if not used immediately.

Solution 4

	stock	for 100 ml
50 mM Tris base	1 M	5 ml
5 mM $MgCl_2$	1 M	0.5 ml
40 % glycerol		40 ml
0.1 mM EDTA	0.5 M	20 ul

Add 30 ml of qH_2O, adjust pH to 8.3 with HCl and make up volume to 100 ml. Filter sterilize.