Nuclear and Cytoplasmic Extract

2/2/94 TT

1. Turn on the centrifuge machines and cool the rotors.
2. Prepare buffers. 1-2 ml/15 cm dish of hypotonic buffer and 25-50 ml/15 cm dish of high and low salt buffer are required.
3. Wash the dishes once with PBS(-) (RT).
4. Add 1 ml ice cold PBS and harvest the cells with rubber policeman. Then collect the cells to orange cap tube and centrifuge in a Beckman GS-6 rotor 1,500 rpm, 4C for 5 min.
5. Remove sup. and resuspend ppt with 5 times packed cell volume (pcv) of hypotonic buffer. Spin 1,500 rpm, 4C, 5min.
6. Add 2 volume of original pcv of hypotonic buffer and resuspend the packed cells. Incubate 10 min on ice.
7. Transfer the cells to a glass Dounce homogenizer and homogenize with 10 strokes using pestle B. Check for cell lysis in a microscope. Add more strokes if necessary.
8. Collect the nuclei by centrifuging 15 min in a Beckman GS-6 rotor at 3,750 rpm (= 3210 xg), 4C. Remove sup and save for S-100 (see 9' below).
9. Using graduation of the tube, add 0.5 pnv (packed nuclear volume) of low salt buffer and resuspend with gentle pipetting.
10. In a dropwise fashion, while vortexing gently (setting 2), add 0.5 pnv of high salt buffer. Incubate 30 min on ice, on tilting board. Start preparing dialysis buffer.
11. Centrifuge the sample in Beckman TLS 55 rotor at 19,000rpm (=25,000 xg), 30 min, 4C.
12. Draw off resulting sup and dialyze against 500 volume of dialysis buffer for 3 hrs.
13. Centrifuge the extract in Beckman TLS 55 rotor at 19,000rpm (=25,000 xg) 20 min, 4C. Discard the ppt.
14. Determine the protein concentration of the sup (Bradford) and KCl concentration (conductivity meter). Aliquot into tubes and rapidly freeze in liquid N_2 or dry ice/methanol bath.

Cytoplasmic extraction (S-100).

9'. Add 0.11 vol of 10x cytoplasmic extract buffer.

10'. Centrifuge in Beckman 50 rotor at 40,000 rpm (=100,000 xg), 1 hr, 4C. to step12

Solutions

Add following protease inhibitors and reducing agent to hypotonic buffer, high salt buffer, and low salt buffer immediately before use.

1 mM PMSF (final) 4ul/ml 0.25 M PMSF (in ethanol stock)
1 tablet per 50 ml of solution of Complete Protease Inhibitor (Boehringer-Manheim)

***Hypotonic buffer (for 100 ml)**

10 mM HEPES, pH 7.9 at 4uC	1 M HEPES (pH7.9):	1 ml
1.5 mM $MgCl_2$	1 M $MgCl_2$	150 ul
10 mM KCl	1 M KCl	1 ml
	qH_2O	98 ml

2 M KCl buffer (for 100 ml)

20 mM HEPES, pH 7.9 at 4C	1 M HEPES (pH7.9):	2 ml

1.5 mM $MgCl_2$	1 M $MgCl_2$	150 ul
25% glycerol	glycerol	25 ml
2 M KCl	3 M KCl	66.7 ml
0.2 mM EDTA	0.5 M EDTA	40 ul
	qH_2O	6.11 ml

KCl(-) buffer (for 100 ml)

20 mM HEPES, pH 7.9 at 4uC	1 M HEPES (pH7.9):	2 ml
1.5 mM $MgCl_2$	1 M $MgCl_2$	150 ul
25% glycerol	glycerol	25 ml
0.2 mM EDTA	0.5 M EDTA	40 ul
	qH_2O	73 ml

***High-salt buffer**

20 mM HEPES, pH 7.9 at 4uC
1.5 mM $MgCl_2$
25% glycerol
0.2 mM EDTA — for 1.0 M KCl, mix 1 vol of 2 M KCl buffer and
Desired KCl (0.8, 1.0, 1.2, 1.4, or 1.6 M) — 1 vol of KCl(-) buffer.

***Low-salt buffer**

20 mM HEPES, pH 7.9 at 4uC
1.5 mM $MgCl_2$
25% glycerol
0.2 mM EDTA
0.02 M KCl — Mix 1 vol of 2 M KCl buffer and 99 vol of KCl(-) buffer.

10x Cytoplasmic extract buffer (for 10 ml)

0.3 M HEPES, pH 7.9 at 4C	1 M HEPES (pH7.9)	3 ml
0.03 M $MgCl_2$	1 M $MgCl_2$	300 ul
1.4 M KCl	3 M KCl	4.67 ml
	qH_2O	2.03 ml

Dialysis buffer (for 1 L) Make in a larger beaker to allow vigorous stirring.

20 mM HEPES, pH 7.9 at 4C	1 M HEPES (pH7.9):	20 ml
1.5 mM $MgCl_2$	1 M $MgCl_2$	1.5 ml
20 % glycerol	glycerol	200 ml
100 mM KCl	3 M KCl	33.3 ml
0.2 mM EDTA	0.5 M EDTA	400 ul
	qH_2O	745 ml
1 mM PMSF	174.2 mg/4 ml ethanol or isopropanol, add before cooling the buffer.	
1 mM DTT	154.2 mg	

Ref.: Abmyr and Workman, Current Protocols in Molecular Biology: 12. 1. 1