Isolation of Nuclei from Somatic Cells

1. HeLa Cells, 293T Cells, NT2 Cells

Cell preparation

- harvest cells from flasks as per standard protocol
- spin cells in 50 ml conical tube at 1500 rpm for 10 min at RT
- resuspend cells in 30 ml PBS; take a 50 l sample to determine concentration

Isolation of Nuclei

1. spin cells at 1500 rpm, 10 min, 4°C
2. discard PBS and resuspend cells in 20 ml of ice-cold hypotonic buffer N containing 1 mM PMSF and 1 mM DTT
3. spin cells at 1500 rpm, 10 min, 4°C
4. discard supernatant, resuspend cells in 10-20 vol of ice-cold hypotonic buffer N containing 1 mM DTT and all protease inhibitors
5. incubate cells on ice for 10-60 min (10 min is sufficient for 293T and NT2 cells; >30 min is preferable for HeLa cells)
6. homogenize cells with a glass Dounce pestle B **on ice**: 293 T cells usually require 10-20 strokes, HeLa cells usually require >100 strokes, NT2 cells require 10 gentle strokes
7. monitor cell lysis under phase contrast microscope (40x); nuclei appear a lot more refringent than cells. All cells should be lysed, but the nuclei should remain intact.
8. when cells are lysed, add 125 µl of 2 M sucrose solution per ml of lysate; mix well by inversion
9. centrifuge the lysate at 1000 rpm in 15 ml conical tubes, 10 min, 4 C, in swinging bucket rotor
10. nuclei are in the pellet and are clean; decant supernatant and resuspend nuclei in a 5-10 ml of ice-cold buffer N
11. centrifuge nuclei at 1,000 rpm, 10 min, 4°C
12. decant supernatant and resuspend nuclei in either desired volume of ice-cold buffer N or in freezing medium (see below for freezing protocol).

Freezing of nuclei

Freezing medium: 70% glycerol in buffer N

To prepare 50 ml of freezing medium, mix 15 ml of buffer N containing 1 mM DTT and 1 mM PMSF with 35 ml of pure glycerol. Stir on a stir plate until the mixture is homogenous. This medium can be stored *frozen*.

Protocol:

1. resuspend the pelleted nuclei obtained in step 11 above into desired volume of freezing buffer to obtain a concentration of 10^6 nuclei / ml. Replace the tube on ice as soon as possible
2. aliquot in 500 μl into 1.5-ml eppendorf tubes. This will give $5x10^5$ nuclei/aliquot, which is more than sufficient for most of our applications.
3. freeze aliquots directly in the -80 C freezer and store at –80°C. Nuclei can also be frozen in a methanol-dry-ice bath and stored at -80°C. Freezing in liquid nitrogen should be avoided.

Thawing and washing of frozen nuclei

1. take out 1 tube of frozen nuclei (500 μl) and thaw it at room temp; place on ice
2. dilute with 1 ml of ice-cold buffer N
3. mix well but gently with a 1000 μl pipette tip
4. centrifuge at 3,000 rpm for 15 min in the swingout rotor
5. discard supernatant and resuspend nuclei in 500μl ice-cold buffer N
6. centrifuge at 3,000 rpm for 10 min in swingout rotor
7. resuspend pellet in ice-cold buffer N to the desired concentration; keep on ice until use

2. Isolation of Nuclei from Lymphoid Cells (T cells, Bjab, Reh, KE37)

- collect cells by centrifugation at 400 g (1,500 rpm), RT, 10 min in 50 ml tubes
- resuspend cells in 5-10 ml PBS and if needed, pool the cells into 1 or 2 tubes
- add PBS to each tube to 50 ml
- centrifuge cells for 10 min at 1,500 rpm, RT
- resuspend cells in PBS to 10 ml final volume
- take a 50 μl aliquot and use to count the cells
- centrifuge the cells as above and remove as much of the PBS as possible
- resuspend cells into cold hypotonic buffer (see below) at $50x10^6$ cells / ml
- add NP-40 to 0.5% (5 μl/ml buffer) and mix well but gently
- centrifuge cell lysate at 1,000 rpm (400 g) for 7 min at 4°C
- carefully aspirate and discard the supernatant. The pellet contains clean nuclei.
- wash nuclei by resuspending them in 10 ice-cold buffer N and centrifuge at 1,500 rpm, 10 min, 4°C
- resuspend nuclei in buffer N or freezing medium and keep on ice until use. Alternatively, proceed with freezing protocol.

Thawing and washing of frozen nuclei

1. take out 1 tube of frozen nuclei (500 μl) and thaw it at room temp; place on ice
2. dilute with 1 ml of ice-cold buffer N
3. mix well but gently with a 1000 μl pipette tip
4. centrifuge at 3,000 rpm for 15 min in the swingout rotor
5. discard supernatant and resuspend nuclei in 500μl ice-cold buffer N
6. centrifuge at 3,000 rpm for 10 min in swingout rotor
7. resuspend pellet in ice-cold buffer N to the desired concentration; keep on ice until use

Hypotonic Buffer N

For 100 ml:

Hepes pH 7.5	10 mM	1 ml	of 1 M stock
$MgCl_2$	2 mM	200 µl	of 1 M stock
KCl	25 mM	800 µl	of 3 M stock
H_2O		98 ml	

This stock solution can be stored frozen between uses.

Before use, add:

DTT	1 mM, from 1 M stock (-20°C); 1 µl/ml
PMSF	1 mM, from 100 mM stock; 10 µl/ml
CAL mix*	10 µg/ml each - add before use, from cocktail stock solution
Pepstatin A*	10 µg/ml – add 10 µl stock per ml

* Alternatively, use a broad range protein inhibitor cocktail.

Buffer N

For 100 ml:

Hepes pH 7.5	10 mM	1 ml	of 1 M stock
$MgCl_2$	2 mM	200 µl	of 1 M stock
KCl	25 mM	800 µl	of 3 M stock
Sucrose	250 mM	12.5 ml	of 2 M stock in H_2O
H_2O		85 ml	

This stock solution can be stored frozen between uses.

Before use, add:

DTT	1 mM, from 1 M stock (-20°C); 1 µl/ml
PMSF	1 mM, from 100 mM stock; 10 µl/ml
CAL mix*	10 µg/ml each - add before use, from cocktail stock solution
Pepstatin A*	10 µg/ml – add 10 µl stock per ml

* Alternatively, use a broad range protein inhibitor cocktail.

Optional, an only if when it is difficult to isolate clean nuclei free of microfilaments

Cytochalasin D§	*1 µg/ml, from 1 mg/ml stock (-20°C); 1 µl/ml*

§or cytochalasin B 10 µg/ml, from 1 mg/ml stock (-20°C); 10 µl/ml

Freezing medium (70% glycerol in buffer N)

To prepare 50 ml of freezing medium, mix 15 ml of buffer N containing 1 mM DTT and 1 mM PMSF with 35 ml of pure glycerol. Stir on a stir plate until the mixture is homogenous. This medium can be stored *frozen*.