Isolation of nuclei from tissue culture cells

The nucleus is the largest and densest organelle of a mammalian cell. It can be easily purified by sedimentation in aqueous and non-aqueous solutions. The purified nuclei can be directly used in downstream assays or to isolate nuclear protein complexes, chromatin, histones, nuclear RNA, or nucleoli.

In *Alternative A*, cells are swollen and ruptured by applying gentle mechanical stress. In *Alternative B*, the cellular membrane is solubilized with a mild detergent such as Triton™ X-100 or IGEPAL® CA-630 which serves as a substitute for Shell's discontinued Nonidet P-40. Although intact nuclei are inert towards diffusion of macromolecules larger than 30–40 kDa, even mild treatment can lead to partially disrupted nuclear membranes and result in increased diffusion and potential aggregation of nuclei.

Risk assessment

– Work with human-derived material or transgenic cell lines (BSL-2)

▷ Wear gloves, safety glasses, lab coat

☐ Collect and dispose waste after inactivation as REGULATED MEDICAL WASTE

Reviewed: Feb 22, 2025

Procedures

Preparation of cells

☐ Phosphate-buffered saline, pH 7.4

☐ 100 × Inhibitor cocktail, 1 mL ⟨*R*⟩

(1.) Start with $1{\times}10^6$–$1{\times}10^9$ cells. When working with frozen cell pellets, thaw on ice. Approximate the packed cell volume; this is 1 vol. Typically, $1{\times}10^9$ cells amount to 1–2 mL cell volume.

Critical: Keep buffer volumes to a minimum to maintain high protein concentrations.

(2.) Wash cells twice with 5 vol ice-cold PBS with inhibitors. Try to obtain a suspension free of visible clumps. Collect the cells at 400 × *g* for 5 min at 4 °C. 10 min

A › ***Detergent-free isolation of nuclei***

☐ Dounce homogenizer, loose pestle

☐ Swelling buffer, 10 mL ⟨*R*⟩

(1.) Resuspend the cell pellet in 5 vol ice-cold 1 × hypotonic swelling buffer with inhibitors. Ensure the suspension is free of clumps. Incubate for 10 min on ice to swell the cells. 10 min

(2.) Collect the cells by centrifugation at 400 × *g* for 10 min at 4 °C, discard the supernatant. 10 min

(3.) Resuspend in 2 vol (at least 0.4 mL) swelling buffer. Transfer to a pre-chilled Dounce homogenizer.

Note: When choosing the proper size for the homogenizer, be sure the total volume of the cell suspension does not exceed one-third of the volume of the lower chamber of the glass cylinder.

Critical: Do not vortex! Great care must be exercised throughout this procedure. Repeated pipetting and centrifugation will result in compromised nuclei which are very susceptible to leaking and disruption.

(4.) Using a type A (or "loose") pestle, lyse the cells by 10–15 strokes. Apply even pressure and deliberate strokes for uniform lysis. Avoid the formation of bubbles and foam. Repeat until lysis is complete.

Quality assurance: Monitor lysis by removing 5–10 µL lysate. Mix with an equal volume of 0.4% Trypan blue; view under a microscope. Stop homogenization when 95% of cells lost their cytoplasmic membrane and nuclei still remain intact.

(5.) Transfer to a centrifuge tube, pellet broken cells and nuclei at 400 × *g* for 5 min at 4 °C. Keep the supernatant as cytoplasmic fraction if desired.

Quality assurance: The nuclear pellet should be white as opposed to the off-white/beige cellular pellet.

Note: 400–600 × *g* is sufficient to pellet nuclei and will not pellet other organelles.

(6.) Wash once in 1.5 vol hypotonic swelling buffer to remove cell membranes.

(7.) Resuspend the nuclei in the desired buffer for downstream experiments.

[DK19]

B › ***Isolation of nuclei in isosmotic sucrose***

- ☐ Sucrose buffer, 10 mL ⟨*R*⟩
- ☐ 10% Triton™ X-100

(1.) Wash the cell pellet twice in 2–5 vol ice-cold isosmotic sucrose buffer with inhibitors.

(2.) Resuspend in 1–2 vol ice-cold sucrose buffer with inhibitors. Ensure the suspension is free of clumps. Admix an equal volume of sucrose buffer containing 0.6% Triton X-100. Incubate for 10 min on ice. 10 min

> ***Critical:*** If no a single cell suspension is obtained at this point, cell lysis will not be homogenous. Avoid shearing membranes and organelles by pipetting very gently.

(3.) Pellet at 400 × *g* for 5 min at 4 °C. If desired, keep the supernatant as cytoplasmic fraction.

(4.) Wash twice with 2–5 vol sucrose buffer to remove cell membranes.

(5.) Resuspend the nuclei in the desired buffer for downstream experiments.

[QZW+23]

Storage of isolated nuclei

(1.) Resuspend the nuclei in isosmotic sucrose buffer; snap-freeze in liquid nitrogen or a dry ice–ethanol bath. Store at −80 °C for up to two years. Do not refreeze.

Analyses

- ○ Verify the integrity of nuclei and the absence of free chromatin by staining with Trypan blue.
- ○ Determine the nucleic acid content spectrophotmetrically. Dilute a sample 20–100 × in 2 M NaCl and record the absorption at 260 nm and 280 nm; the A260/A280 ratio should range 1.6–1.7. Adjust the concentration to an A260 of 40 or higher (800 ng/μL DNA) to minimize the osmotic pressure.

  > ***This is why:*** The high salt concentration will disintegrate the nuclear membrane, releasing the chromatin, and disrupt protein–nucleic acid complexes for the absorption measurement.

- ○ Prepare samples for SDS-PAGE correpsonding to 2×10^5 cells (or 20 μg protein) for the total, cytoplasmic, and nuclear fraction. Immunostain for tubulin (55 kDa; cytoplasm), and/or histone H3 (15 kDa; chromatin) to demonstrate successful fractionation.

Troubleshooting

Preparation of cells

In Step 2:

– Cells clump together.

  ◦ If unable to disrupt the cell pellet, a short pulse of sonication can be attempted to separate aggregates.

Detergent-free isolation of nuclei

In Step 4:

– Incomplete cell lysis after repeated douncing.

  ◦ Use type B (or "tight") pestle instead.

In Step 5:

– No nuclear pellet.

  ◦ Nuclear pellets isolated from small cell numbers (less than $1{\times}10^6$ cells), are often very hard or impossible to see. Centrifuge for 30 min to increase visibility. Proceeding without a visible pellet often also yields decent results.

Isolation of nuclei in isosmotic sucrose

In Step 2:

– Incomplete cell lysis or leaking nuclei in the presence of Triton™ X-100.

  ◦ Vary detergent concentration, typically within 0.1–0.5% Triton™ X-100. Try alternative detergents such as IGEPAL® CA-630. Avoid Tergitol-type NP-40 ("Nonident P-80"; CAS 9016-45-9) as it may dissolve the nuclear membrane.

Recipes

Inhibitor cocktail, 100×

Amount	Ingredient		Stock	Final
20 µL	Phenylmethylsufonyl fluoride (PMSF) ◇ R0035		100 mM	2 mM
370 µL	Leupeptin · HCl	[24125-16-4]	25 g/L	2.0 mM
411 µL	Pepstatin A	[26305-03-3]	25 g/L	1.5 mM
500 µL	Aprotinin	[9087-70-1]	10 g/L	80 µM
100 µL	β-Glycerophosphate	[13408-09-8]	1 M	100 mM
0.5 µL	Trichostatin A	[58880-19-6]	20 mM	10 µM
To 1 mL	Water, reagent-grade			

Add or omit inhibitors as needed. ***Note:*** This inhibitor cocktail preserves histone acetylation and phosphorylation marks.

100× Inhibitor cocktail

200 µM PMSF, 20 µM Leupeptin, 15 µM Pepstatin A, 0.8 µM Aprotinin, 10 mM β-Glycerophosphate, 1 µM Trichostatin A *[At 1× dilution]*

Date: Sign: R0090

Swelling buffer, pH 7.4

Amount	Ingredient		Stock	Final
100 µL	Tris-Cl, pH 7.4	◇ R0055	1 M	10 mM
33 µL	KCl	◇ R0038	3 M	10 mM
15 µL	$MgCl_2$	◇ R0031	1 M	1.5 mM
5 µL	Dithiothreitol (DTT)	☐ ◇ R0015	1 M	0.5 mM
100 µL	Inhibitor cocktail	☐ ◇ R0090	100×	1×
To 10 mL	Water, reagent-grade			

Add DTT and inhibitor cocktail immediately before use. Store at 4 °C.

Swelling buffer

10 mM Tris-Cl, 10 mM KCl, 1.5 mM $MgCl_2$, ☐ 0.5 mM DTT, ☐ 1× Inhibitor cocktail, pH 7.4

Date: Sign: R0091

Sucrose buffer, pH 7.4

Amount	Ingredient		Stock	Final
150 µL	Tris-Cl, pH 7.4	R0055	1 M	15 mM
1 000 µL	Sucrose	R0066	2.5 M	250 mM
200 µL	KCl	R0038	3 M	60 mM
30 µL	NaCl	R0046	5 M	15 mM
50 µL	$MgCl_2$	R0031	1 M	5 mM
10 µL	Dithiothreitol (DTT)	☐ R0015	1 M	1 mM
100 µL	Inhibitor cocktail	☐ R0090	100 ×	1 ×
To 10 mL	Water, reagent-grade			

Add DTT and inhibitor cocktail immediatly before use. Store at 4 °C.

Sucrose buffer

15 mM Tris-Cl, 250 mM Sucrose, 60 mM KCl,
15 mM NaCl, 5 mM $MgCl_2$, ☐ 1 mM DTT,
☐ 1 × Inhibitor cocktail, pH 7.4

Date: Sign: R0092

List of references

J. DeCaprio and T.O. Kohl, *Cold Spring Harbor Protoc.* **2019**(7), (2019).

Y. Qin, Y. Zhou, K. Wang, J. Gu, Z. Xiong, W. Zhang, and Y. Chen, *Biol. Res.* **56**(1), 18 (2023).

Change log

2020-03-22	Shany Koren-Hauer	Initial version (Detergent-free isolation of nuclei).
2022-07-09	Benjamin C. Buchmuller	Adaptation as SOP. Initial version (Isolation in isosmotic surcose).
2024-04-21	Benjamin C. Buchmuller	Updated inhibitor cocktail.
2025-02-22	Benjamin C. Buchmuller	Corrected references.