Nuclear extraction and fractionation of chromatin-associated proteins

The eukaryotic nucleus contains distinct biochemical environments that regulate access to the genetic information and modulate protein interactions. These include the nucleosol, euchromatin, heterochromatin, and insoluble structures such as nuclear bodies, the nucleolus, and the nuclear matrix.

Many nuclear proteins associate with DNA through charge-based interactions. Soluble nucleosolic proteins, including transcription factors and chromatin regulators, are already released under low salt conditions. More tightly bound proteins can be extracted in a single step at intermediate salt concentrations (total chromatin), or fractionated sequentially with increasing salt to distinguish between euchromatin and heterochromatin. Compacted chromatin and nucleosome-associated proteins that remain insoluble after high salt extraction become accessible through partial or complete micrococcal nuclease (MNase) digestion. The remaining pellet can be further processed by sonication or acidic extraction 0026.

The resulting fractions can be used for immunoblotting, co-immunoprecipitation, nucleosome-dipping, or proteomics. This protocol has limited use for profiling transient or dynamic protein–chromatin interactions, which may be disrupted by salt or nuclease treatment.

Begin with isolated nuclei 0001.

Risk assessment

▷ Wear gloves, safety glasses, lab coat

Reviewed: Dec 10, 2023

Procedures

Choosing a nuclear extraction and fractionation workflow

(1.) Choose a strategy for extracting target proteins from subnuclear compartments. If unsure, start with salt fractionation after partial MNase digestion or total chromatin extraction.

Target compartment	Accessibility	Buffer composition	Extraction strategy	Example markers
Nucleosol	High	<150 mM NaCl	Low salt extraction	Sp1, Pol II
Euchromatin	Intermediate	150–300 mM NaCl	Partial MNase digestion	H3K4me3, BRD4
Heterochromatin	Low	>400 mM NaCl	Partial MNase digestion	HP1, H3K9me3
Total chromatin	Varying	>400 mM NaCl	Full MNase digestion	
Insoluble histones	Very low	Not applicable	Acid extraction 0026	Histone H1
Nucleolar proteins	Very low	Not applicable	Sonication	NPM1

Hint: Nuclear extraction buffers use Tris-Cl by default. When planning for mass spectrometry, consider replacing with 10 mM HEPES pH 7.5 or dialyzing the extract. To enhance protein complex stability for immunoprecipitation, add 10–20% glycerol.

Preparing nuclei for extraction and optional MNase digestion

- ☐ Tris-NaCl-KCl, 250 µL ⟨R⟩
- ☐ 200 U/mL Micrococcal nuclease, 50 µL ⟨R⟩
- ☐ 0.5 M Ethylene glycol tetraacetic acid, 10 µL ⟨R⟩

(1.) Start with a nuclei pellet isolated from 1.0×10^6–1.0×10^7 cells. Bring to 1.0×10^7 /mL in TNK buffer. Gently flick the tube to disperse remaining clumps.

Hint: For continuous cell lines such as HeLa, U2OS, or 293T, 1.0×10^6 cells typically yield sufficient material. Primary or finite cell strains may require 1.0×10^7 cells per condition.

Hint: Supplement 10% glycerol to stabilize protein complexes for IP. Sucrose offers but weak protein stabilization.

Critical: The nuclear membrane is fragile! Gentle pipetting is sufficient to disrupt most of the pellet. Clumping after resuspension indicates lysis — discard and repeat. If iso-osmotic sucrose buffer containing Tergitol-type NP-40 (CAS 9016-45-9) was used to prepare nuclei, highly mobile nuclear proteins like HMGB1 may already be lost.

(2.) *Optional:* Supplement the TNK buffer for chromatin digestion with calcium and MNase. Incubate with gentle mixing at 37 °C, and inactivate on ice. See table for common conditions.

Target fraction	MNase	Additives	Treatment	Inactivation
Total chromatin	5.0 U/mL	1 mM $CaCl_2$	20 min at 37 °C	5 mM EGTA
Euchromatin	1.2 U/mL	1 mM $CaCl_2$	10 min at 37 °C	5 mM EGTA

> ***Quality assurance:*** Optimize MNase concentration and time for each cell type. Ideal digestion yields mostly mononucleosomes with minor di-/tri-nucleosome signal. Excessive MNase treatment will chop all nucleosomal DNA down to 10 bp fragments.

> ***This is why:*** EGTA preferentially chelates Ca^{2+} leaving Mg^{2+} in solution, a mediator of many protein-protein interactions.

(3.) Pellet the nuclei at 400 × *g* for 10 min at 4 °C. If MNase was used, keep the supernatant for analysis.

> ***Hint:*** In untreated samples, the supernatant may contain loosely associated proteins from nuclei with ruptured membrane. Discard unless required for analysis.

(4.) Wash the pellet once with 2–5 vol TNK buffer and proceed to extraction.

[TH12]

›› *Nuclear extraction and fractionation*

- ☐ Nuclear extraction buffer, low-salt (LS), 10 mL ⟨*R*⟩
- ☐ Nuclear extraction buffer, high-salt (HS), 10 mL ⟨*R*⟩

(1.) Prepare buffers with defined NaCl concentrations by mixing LS and HS nuclear extraction buffers:

Nuclear extraction buffer	0 mM NaCl	75 mM NaCl	150 mM NaCl	300 mM NaCl	400 mM NaCl
Low-salt	1.5 mL	0.2 mL	0.4 mL	0.8 mL	1.0 mL
High-salt	0.0 mL	1.4 mL	1.2 mL	0.8 mL	0.5 mL

(2.) Resuspend the nuclear pellet in 100 µL extraction buffer per $1.0{\times}10^7$ nuclei. Add fresh inhibitors.

> ***Critical:*** Use chilled buffers. Add protease inhibitors just before use to prevent degradation.

(3.) Incubate on ice for 30 min. For volumes over 100 µL, rotate at 4 °C. 30 min

(4.) Separate soluble fraction by centrifugation at 400 × *g* for 10 min at 4 °C. Collect the supernatant.

(5.) Repeat the extraction using buffers with increasing salt concentration on the remaining pellet. Analyze and pool fractions as needed.

(6.) The remaining chromatin pellet can be collected at 16 000 × *g* for 40 min at 4 °C and resuspended for further processing such as acidic extraction or sonication.

> ***This is why:*** Chromatinized histones remain insoluble even at 400 mM salt unless nuclei were fully digested with MNase.

[HAW17; KNC20]

Analyses

- ○ Prepare samples from each fraction for SDS-PAGE and immunoblotting. Load material corresponding to 2.0×10^5 cells or 10–20 µg total protein per lane.
- ○ Probe for compartment-specific markers to assess fractionation quality: Tubulin (55 kDa; cytoplasm), transcription factor Sp1 (80 kDa; nucleosol), and/or histone H3 (15 kDa; chromatin).

> ***Resource:*** The Nuclear Protein Database (https://webapps.igc.ed.ac.uk/npd/) by Bickmore and Sutherland (2002) provides an overview of the diversity of many subnuclear compartments.

- ○ *Optional:* Subject select fractions to immunoprecipitation, mass spectrometry, or nucleosome profiling.

> ***Critical:*** If samples contain high salt or detergent concentrations, exchange the buffer by dialysis before LC-MS/MS.

Recipes

Tris-NaCl-KCl (TNK), pH 7.6

Amount	Ingredient		Stock	Final
0.5 mL	Tris-Cl, pH 7.4	R0055	1 M	10 mM
1.0 mL	Potassium chloride (KCl)	R0038	3 M	60 mM
150 µL	Sodium chloride (NaCl)	R0046	5 M	15 mM
To 50 mL	Water, reagent-grade			

Tris-NaCl-KCl (TNK)

10 mM Tris-Cl, 60 mM KCl, 15 mM NaCl, pH 7.6

Date: Sign: R0143

Micrococcal nuclease (MNase), 200 U/mL

Amount	Ingredient	Stock	Final
50 U	Micrococcal nuclease, from *Staphylococcus aureus* [9013-53-0]	100–300 U/mg	0.2 U/µL
20 µL	Sodium phosphate, pH 7.0	1 M	5 mM
To 4 mL	Water, reagent-grade		

Dispense into 100 µL aliquots. Stable for 1 year at −20 °C. ***Note:*** Multiple freeze-thaw cycles are tolerated.

200 U/mL Micrococcal nuclease (MNase)

0.2 U/µL Micrococcal nuclease, 5 mM Sodium phosphate

Expiry: Sign: R0144

Ethylene glycol tetraacetic acid (EGTA), 0.5 M

Amount	Ingredient		Stock	Final
1.90 g	Egtazic acid	[67-42-5]	380.35 g/mol	0.5 M
To 10 mL	Water, reagent-grade			

EGTA will not dissolve well until pH 8; titrate in with NaOH.

0.5 M Ethylene glycol tetraacetic acid (EGTA)

WARNING

Skin irritation; Eye irritation

Date: Sign: R0145

Nuclear extraction buffer, low-salt (LS), pH 7.4

Amount	Ingredient		Stock	Final
100 µL	Tris-Cl	R0055	1 M	10 mM
20 µL	$MgCl_2$	R0031	1 M	2 mM
40 µL	EGTA	R0145	0.5 M	2 mM
150 µL	Triton™ X-100	☐ R0057	10%	0.1%
100 µL	Inhibitor cocktail	☐ R0090	100 ×	1 ×
To 10 mL	Water, reagent-grade			

Filter sterilize. Store at room temperature. ***Hint:*** The addition of detergent is optional, but facilitates resuspension of the chromatin fraction. To enhance protein complex stability for downstream IP, supplement the buffer with 10–20% glycerol.

Nuclear extraction buffer, low-salt (LS)

10 mM Tris-Cl, 2 mM $MgCl_2$, 2 mM EGTA,
☐ 0.1% Triton™ X-100, ☐ 1 × Inhibitor cocktail,
pH 7.4

Date: Sign: R0146

Nuclear extraction buffer, high-salt (HS), pH 7.4

Amount	Ingredient		Stock	Final
100 µL	Tris-Cl	R0055	1 M	10 mM
2.4 mL	Sodium chloride (NaCl)	R0046	5 M	0.6 M
20 µL	$MgCl_2$	R0031	1 M	2 mM
40 µL	EGTA	R0145	0.5 M	2 mM
150 µL	Triton™ X-100	☐ R0057	10%	0.1%
100 µL	Inhibitor cocktail	☐ R0090	100 ×	1 ×
To 10 mL	Water, reagent-grade			

Filter sterilize. Store at room temperature. ***Hint:*** The addition of detergent is optional, but facilitates resuspension of the chromatin fraction. To enhance protein complex stability for downstream IP, supplement the buffer with 10–20% glycerol.

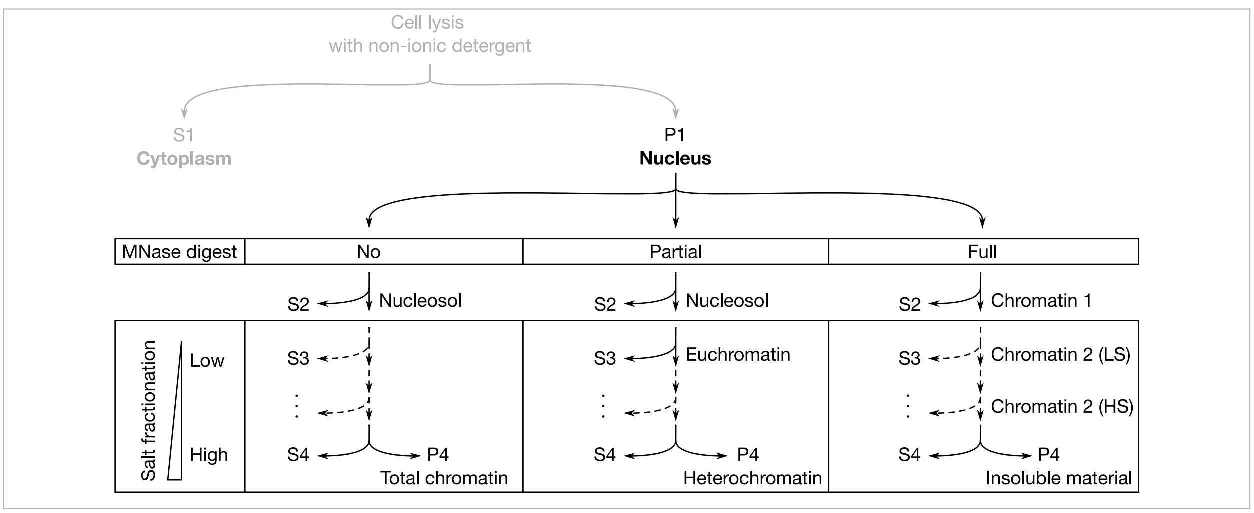
Nuclear extraction buffer, high-salt (HS)

10 mM Tris-Cl, 0.6 M NaCl, 2 mM $MgCl_2$,
2 mM EGTA, ☐ 0.1% Triton™ X-100,
☐ 1 × Inhibitor cocktail, pH 7.4

Date: Sign: R0147

Resources

Choosing a nuclear extraction and fractionation workflow



In Step 1: Salt extraction alone releases soluble nuclear proteins (S2). Partial MNase digestion improves solubilization of chromatin-bound proteins during salt fractionation. After complete digestion, 20% of total histone H3 and H4 may already appear in the first extract (S2), and up to 70% after the second extract (S3), regardless of salt concentration. Remaining histones in the pellet (P4) require acid extraction.

List of references

W.A. Bickmore and H.G.E. Sutherland, *EMBO J.* **21**(6), 1248—1254 (2002).

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K.A. Khan, M.K. Ng, and P. Cheung, *Front. Cell Dev. Biol.* **8** 331 (2020).

S.S. Teves and S. Henikoff, *Methods Mol. Biol.* **833** 421—432 (2012).

Change log

2023-10-18	Benjamin C. Buchmuller	Adaptation as SOP.
2023-12-10	Benjamin C. Buchmuller	Revised figures; replaced washing step in isosmotic sucrose with commonly used TEK buffer; included useful hints from PMID:32457909.