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.

Intact nucleosomes and their binding partners are released by complete nuclease digestion followed by chelation (*Alternative A*), whereas many nuclear proteins associate with DNA through charge-based interactions and therefore require increasing salt concentration to dissociate (*Alternative B*). 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 high salt after full digestion (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 SOP 0026.

The fractions can be analyzed by immunoblotting, proteomics, or immunoprecipitation of the soluble, salt-extracted fraction SOP 0027 or of the intact nucleosomes SOP 0047. Salt and nuclease disrupt transient protein–chromatin interactions, so none of these fractions is suited to profiling dynamic binding.

Risk assessment

▷ Wear gloves, safety glasses, lab coat

Reviewed: Sep 7, 2026

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 (*Alternative B*) or with the chelator release of intact nucleosomes (*Alternative A*).

Target compartment	Extraction strategy	NaCl	What stays intact	Example markers
Nucleosol	Low-salt extraction	< 150 mM	Complexes	Sp1, Pol II
Euchromatin	Partial MNase digestion, salt extraction	150–300 mM	Complexes	H3K4me3, BRD4
Heterochromatin	Partial MNase digestion, salt extraction	> 400 mM	Partially	HP1, H3K9me3
Total chromatin	Full MNase digestion, salt extraction	> 400 mM	Nothing; nucleosomes dissociate	
Nucleosomes, intact	Full MNase digestion, chelator release	None	Nucleosomes and bound readers	H3, H4
Insoluble histones	Acid extraction SOP 0026	Not applicable	Nothing	Histone H1

Hint: Fraction S1 is the cytoplasm, P1 the nuclei from the nuclei isolation, S2 the first supernatant after the optional digestion, S3 and S4 the successive extracts or the chelator release, and P4 the final pellet.

Critical: Nuclear extraction buffers use Tris-Cl by default. When amine-reactive labeling such as TMT is planned for mass spectrometry, replace Tris with 10 mM HEPES pH 7.5 or exchange the extract into a bicarbonate buffer, since the primary amine of Tris consumes the label.

›› *Preparing nuclei for extraction and optional MNase digestion*

- ☐ Tris-NaCl-KCl, pH 7.6 ◇ R0143
- ☐ 0.5 M Ethylene glycol tetraacetic acid ◇ R0145
- ☐ 200 U/mL Micrococcal nuclease ◇ R0144

(1.) Start with a nuclei pellet SOP 0001 isolated from 1.0×10^6–1.0×10^7 cells, depending on cell type and downstream application. Bring to 1.0×10^7 /mL in TNK buffer. This is 1 vol. Gently flick the tube to disperse remaining clumps.

> ***Hint:*** For continuous cell lines such as HeLa, U2OS, or 293T, 1.0×10^6 cells yield enough for immunoblotting of the fractions; an immunoprecipitation needs about 1.0×10^7 cells. Primary or finite cell strains may require ten times as many cells per condition.

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

> ***Hint:*** This density suits fractions that are read by immunoblot, where dilution costs nothing. Chromatin destined for immunoprecipitation of intact nucleosomes is digested about five times denser and takes its glycerol from the adjustment buffers instead SOP 0047.

> ***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 as indicated. Incubate with gentle mixing at 37 °C, and inactivate on ice.

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

> ***Critical:*** The nuclease concentrations in the table are stated per volume at the density of the previous step, 1.0×10^7 /mL, and correspond to 0.5 U and 0.12 U per 1.0×10^6 cells. Digesting at another density means converting to units per cell first; carrying the units-per-millilitre figure over unchanged under-doses the digest in proportion.

> ***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 as supernatant S2 on ice.

> ***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. Proceed to release for native nucleosomes or nuclear extraction by salt fractionation.

[TH12]

A › *Release of native nucleosomes*

- ☐ Tris/EDTA, pH 8.0 ◇ R0054
- ☐ 100 × Inhibitor cocktail ◇ R0090

(1.) After full MNase digestion, resuspend the pellet in 1 vol Tris/EDTA supplemented on the day with 1 × inhibitor cocktail. Incubate for 30 min on ice, flicking the tube every 10 min. 30 min

> ***This is why:*** Chelation of the divalent cations and the drop of the ionic strength lets the digested chromatin swell and release the nucleosomes that remained in the pellet during the digestion.

(2.) Clear at 13 000 × *g* for 5 min at 4 °C. Transfer the supernatant, fraction S3, to a fresh tube. 5 min

(3.) Set aside 50 µL each of S2 and S3 for the digestion check, or a tenth of the fraction if the digest was run at higher density, then pool the remainder and keep on ice. Proceed with downstream analysis such

as immunoprecipitation of intact nucleosomes SOP 0047, which keeps the two fractions apart until each has been brought to capture conditions.

This is why: S3 usually carries more nucleosomes than S2; together they hold about 75% of the total histone pool. The remainder stays in the insoluble pellet and is recoverable only by acid extraction SOP 0026.

Critical: Do not proceed to salt extraction. Above 400 mM NaCl, linker histone and some H2A–H2B dimers dissociate.

[KNC20]

B › ***Nuclear extraction and fractionation***

☐ Nuclear extraction buffer, low-salt, pH 7.4 R0146 ☐ Nuclear extraction buffer, high-salt, pH 7.4 R0147

(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	1.4 mL	1.2 mL	0.8 mL	0.5 mL
High-salt	0.0 mL	0.2 mL	0.4 mL	0.8 mL	1.0 mL

(2.) Resuspend the nuclear pellet in 100 µL extraction buffer per 1.0×10^{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.

Critical: Exchange the buffer of salt-extracted fractions before LC-MS/MS by dialysis or a desalting spin column; if Triton X-100 was included, it needs a dedicated cleanup such as suspension trapping or paramagnetic-bead (SP3) sample preparation.

[HAW17; KNC20]

Analyses

○ Assess fractionation by SDS-PAGE. Load material equivalent to 2.0×10^{5} cells or 10–20 µg total protein per lane from every fraction and from the final pellet, resolve by SDS-PAGE SOP 0007, blot SOP 0008.

○ Immunostain SOP 0009 for compartment markers.

Quality assurance: Tubulin (55 kDa) must be absent from every nuclear fraction; its presence marks cytoplasmic carry-over from the nuclei preparation. Sp1 (80 kDa) should show in the low-salt extract only. Histone H3 (15 kDa) must remain in the pellet after salt fractionation without digestion (***Alternative B***), and shift into the salt extracts after full digestion. Upon chelator release (***Alternative A***), H3 is detected in the pooled S2 and S3 and no more than a quarter of the total H3 is left in the pellet.

Resource: The Nuclear Protein Database (https://webapps.igc.ed.ac.uk/npd/) by Bickmore and Sutherland (2002) lists markers for other subnuclear compartments.

○ *Optional:* Check the MNase digestion against the target fragment size. Digest the 50 µL aliquots of S2 and S3 with proteinase K (1 U per microgram DNA; 56 °C, 20 min), purify the DNA SOP 0024, and resolve on a 1.2–1.5% agarose gel SOP 0032.

Quality assurance: Partial digestion typically shows a ladder of up to five nucleosomes. Full digestion must show a mononucleosome band at about 150 bp with a minor dinucleosome band. A smear below 150 bp indicates over-digestion; a ladder extending well beyond the trinucleosome after a full digestion predicts poor recovery in S2 and S3.

Materials

Preparing nuclei for extraction and optional MNase digestion

Tris-NaCl-KCl ⟋ R0143, pH 7.6

Micrococcal nuclease ⟋ R0144, 200 U/mL, Stable for 1 year at −20 °C.

Ethylene glycol tetraacetic acid ⟋ R0145, 0.5 M

Release of native nucleosomes

Tris/EDTA ⟋ R0054, pH 8.0

Inhibitor cocktail ⟋ R0090, 100 ×

Nuclear extraction and fractionation

Nuclear extraction buffer, low-salt ⟋ R0146, pH 7.4, Store at room temperature.

Nuclear extraction buffer, high-salt ⟋ R0147, pH 7.4, Store at room temperature.

Recipes

Tris-NaCl-KCl (TNK), pH 7.6

Amount	Ingredient		Stock	Final
0.5 mL	Tris hydrochloride (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

No GHS hazards at the indicated concentrations

☐ Flush to drain with excess water

Date: Sign: R0143

Micrococcal nuclease (MNase), 200 U/mL

Amount	Ingredient		Stock	Final
800 U	Micrococcal nuclease, from *Staphylococcus aureus*	[9013-53-0]	100–300 U/mg	0.2 U/µL
200 µL	Sodium phosphate buffer, pH 7.0	⟋ R0224	100 mM	5 mM
To 4 mL	Water, reagent-grade			

Dispense into 100 µL aliquots. Stable for 1 year at −20 °C. ***Critical:*** Nuclease activity requires 1–5 mM Ca^{2+} and less than 100 mM monovalent salt. ***Note:*** Multiple freeze-thaw cycles are tolerated.

200 U/mL Micrococcal nuclease (MNase)

0.2 U/µL Micrococcal nuclease, 5 mM Sodium phosphate buffer

No GHS hazards at the indicated concentrations

☐ Hold for EHS review before disposal

Date: 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)

No GHS hazards at the indicated concentrations

☐ Dilute below the discharge limit
☐ Flush to drain with excess water

Date: Sign: R0145

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

Amount	Ingredient		Stock	Final
100 µL	Tris hydrochloride (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
100 µL	Triton™ X-100	□ R0057	10%	0.1%
	Skin irritation (H315); Serious eye damage (H318); Aquatic toxicity (long-term) (H411)			
100 µL	Inhibitor cocktail	□ R0090	100 ×	1 ×
	Flammable liquid (H225); Serious eye irritation (H319); Reproductive toxicity (H361)			
To 10 mL	Water, reagent-grade			

Filter sterilize. Store at room temperature. ***Critical:*** Replace Tris-Cl with HEPES when amine-reactive labeling such as TMT is planned for mass spectrometry; the primary amine of Tris consumes the label. ***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

WARNING

Reproductive toxicity

□ Collect as HAZARDOUS WASTE
□ DO NOT wash into sewer

Date: Sign: R0146

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

Amount	Ingredient		Stock	Final
100 µL	Tris hydrochloride (Tris-Cl)	R0055	1 M	10 mM
1.2 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
100 µL	Triton™ X-100	□ R0057	10%	0.1%
	Skin irritation (H315); Serious eye damage (H318); Aquatic toxicity (long-term) (H411)			
100 µL	Inhibitor cocktail	□ R0090	100 ×	1 ×
	Flammable liquid (H225); Serious eye irritation (H319); Reproductive toxicity (H361)			
To 10 mL	Water, reagent-grade			

Filter sterilize. Store at room temperature. ***Critical:*** Replace Tris-Cl with HEPES when amine-reactive labeling such as TMT is planned for mass spectrometry; the primary amine of Tris consumes the label. ***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

WARNING

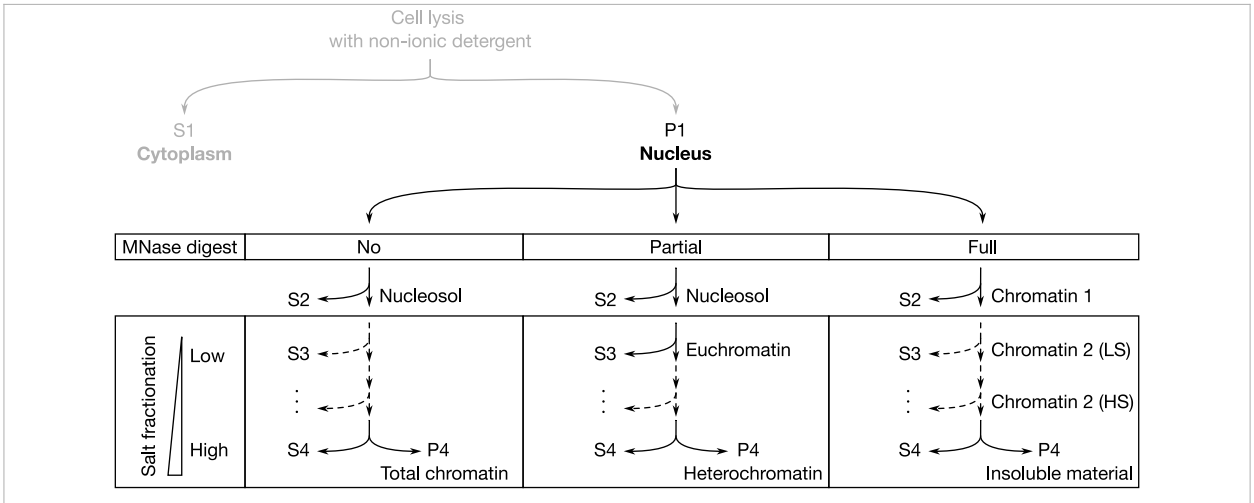
Reproductive toxicity

□ Collect as HAZARDOUS WASTE
□ DO NOT wash into sewer

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).

C. Herrmann, D.C. Avgousti, and M.D. Weitzman, *Bio-protocol* **7**(6), e2175 (2017).

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.
2026-05-22	Benjamin C. Buchmuller	Fix HS NaCl amount (2.4 → 1.2 mL) and Triton X-100 amounts in LS+HS (150 → 100 µL); swap inverted LS/HS rows in the downstream NaCl-mixing table.
2026-05-27	Benjamin C. Buchmuller	Fix MNase recipe amount (50 → 800 U) to match the 0.2 U/µL final in 4 mL.
2026-09-07	Benjamin C. Buchmuller	Added the native nucleosome release after full MNase digestion (chelator, no salt step; pool with the digestion supernatant) for the native chromatin immunoprecipitation protocol. Release conditions from Khan et al., 2020 (Front. Cell Dev. Biol. 8:331).