Nuclear extraction and fractionation of chromatin-associated proteins

Risk assessment

▷ Wear gloves, safety glasses, lab coat



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This is a bench card. Full protocol available online.

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	Ionic strength	Largest unperturbed unit	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	Individual proteins	HP1, H3K9me3
Total chromatin	Full MNase digestion, salt extraction	> 400 mM	Individual proteins	Not applicable
Nucleosomes, intact	Full MNase digestion, chelator release	None	Nucleosomes with bound readers	H3, H4
Insoluble histones	Acid extraction SOP 0026	Not applicable	Histones, denatured	Histone H1

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*

(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.

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.

(3.) Pellet the nuclei at 400 × *g* for 10 min at 4 °C. If MNase was used, keep as supernatant S2 on ice.

(4.) Wash the pellet once with 2–5 vol TNK buffer. Proceed to release for native nucleosomes or nuclear extraction by salt fractionation.

A › *Release of native nucleosomes*

(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

(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.

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

B › *Nuclear extraction and fractionation*

(1.) Prepare buffers with defined NaCl concentrations by mixing the low-salt and high-salt 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.

> ***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.

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