Nuclear extraction and fractionation of chromatin-associated proteins

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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 SOP 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 SOP 0001.

This is a bench card. Full protocol available online.

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 SOP 0026	Histone H1
Nucleolar proteins	Very low	Not applicable	Sonication	NPM1

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

- ☐ R0143 Tris-NaCl-KCl, 250 µL
- ☐ R0144 200 U/mL Micrococcal nuclease, 50 µL
- ☐ R0145 0.5 M Ethylene glycol tetraacetic acid, 10 µL

(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.

> ***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.

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

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

›› *Nuclear extraction and fractionation*

☐ R0146 Nuclear extraction buffer, low-salt (LS), 10 mL ☐ R0147 Nuclear extraction buffer, high-salt (HS), 10 mL

(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 1.0×10^7 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.

List of references

Recipe (available online) Resources (available online) Notes (available online)