Immunoprecipitation of nucleosomes from native chromatin

Pulling down a nucleosome from native chromatin can expose which modifications accompany the probed mark or affinity tag in *cis*, which marks the other histone copies carry in *trans*, or which histone variants, reader proteins, and DNA sequences are present. Typically, the pull-down is analyzed by immunoblotting, mass spectrometry, or next-generation sequencing.

After nuclei are isolated SOP 0001-B and digested to mononucleosomes SOP 0025, the solubilized chromatin is brought to an ionic strength and detergent content tolerated by the capture reagent. Then, the nucleosomes are captured on antibody-loaded Protein A/G beads, on antibody-coupled resins (anti-FLAG, anti-HA), or on nanobody-coupled resins (V5-Trap, GFP-Trap, Spot-Trap). Quantities are given for V5-tagged histone H3 captured on V5-Trap. Plan one day for digestion and adjustment, an overnight capture, and a morning for washes and elution.

Risk assessment

- Calyculin A is an ACUTE TOXIN
- Work with human-derived material or transgenic cell lines (BSL-2)

▷ Wear gloves, safety glasses, lab coat

☐ Collect as HAZARDOUS WASTE

☐ Dispose waste after inactivation as REGULATED MEDICAL WASTE



Reviewed: Nov 1, 2026

Procedures

Planning the immunoprecipitation

(1.) Per immunoprecipitation, grow one confluent 15 cm dish, about 1.0×10^7 cells. Scale digestion and buffer volumes with cell number.

> ***Hint:*** One dish delivers about 1 µg tagged H3, well within the 15 µg capacity of 25 µL slurry.

(2.) For a tagged histone, choose a pre-conjugated resin; for an untagged variant or a modification, load 0.5–5.0 µg of an IP-validated antibody onto Protein A/G beads SOP 0027.

> ***Hint:*** Antibody-loaded Protein A/G beads shed heavy and light chain (50 kDa and 25 kDa) upon SDS elution; crosslink the antibody or elute natively when reader proteins matter. Nanobody traps shed neither.

> ***Resource:*** Validate each antibody lot by immunoblot and peptide binding per the ENCODE guidelines (https://www.encode-project.org/). Peptide-array data are available at http://www.histoneantibodies.com/ which also accepts submissions.

> ***Critical:*** On a mutant histone, epitopes for nearby marks may span the mutation. Check the immunogen peptide and confirm a lost signal with a second antibody against a different peptide or by mass spectrometry.

(3.) Decide whether to solubilize the chromatin with micrococcal nuclease (MNase) or a universal nuclease. Use MNase for DNA sequencing and whenever marks or partners must be assigned to a single nucleosome.

> ***Hint:*** Micrococcal nuclease releases about 150 bp of DNA, against the 200–1 000 bp of a sonicated crosslinked preparation [Abc26]. A universal nuclease leaves DNA fragments of undefined length, but digests can be performed directly in IP buffer within 1 h without buffer adjustment, accelerating the protocol.

> ***Hint:*** Nucleosomes can rearrange while the chromatin is digested, so where exact positions matter, run a crosslinked capture alongside as the control for it [Abc26]; the crosslinking chemistry is in SOP 0027.

(4.) *Critical:* Plan a control capture with the same input, bead lot, and handling: for a tag, use lysate from the parental line without the tagged histone, grown and harvested in parallel, on the same resin; for an antibody, use an isotype IgG on the same Protein A/G beads.

›› *Harvest and isolation of nuclei*

☐ Harvest buffer ⟋ R0037 ☐ Inhibitor supplement ⟋ R0239

(1.) Add the inhibitors to preserve the histone marks of interest. On the day, combine the stocks into about 1 mL of 50 × supplement and add it to every buffer from the harvest wash to the last bead wash, at 20 µL/mL, or 40 µL/mL for the 2 × adjustment buffers. Add PMSF separately:

Inhibitor	Stock	Volume	Final	Protects
Calyculin A ⟋ R0239	100 µM	25 µL	50 nM	Serine and threonine phosphorylation (PP1, PP2A)
β-Glycerophosphate ⟋ R0164	1 M	500 µL	10 mM	Serine and threonine phosphorylation (bulk substrate)
Trichostatin A ⟋ R0165	20 mM	2.5 µL	1 µM	Lysine acetylation (zinc-dependent HDACs)
Nicotinamide ⟋ R0166	1 M	500 µL	10 mM	Lysine acetylation (sirtuins)
PMSF ⟋ R0035	100 mM	separate	1 mM	Serine proteases; add 10 µL/mL at each buffer change

Critical: Keep every buffer up to the EGTA stop free of chelators, which inactivate both nucleases. Keep sodium fluoride below 5 mM as well: the digestion buffer already carries about 75 mM monovalent salt, and the 10–50 mM at which fluoride is usually used would take it past the salt the nuclease tolerates 🗎 SOP 0025.

This is why: Lysine methylation needs no inhibitor. The JmjC demethylases require iron, 2-oxoglutarate, and ascorbate, none of which these buffers supply [TFE+06]; the exception is H3 lysine 4 mono- and dimethylation, whose LSD1-family demethylases are flavin-dependent amine oxidases and need no added cofactor [SLM+04].

(2.) Harvest the cells into inhibitors: aspirate the medium, wash the dish twice with ice-cold harvest buffer, scrape the cells in the second wash, and pellet at 400 × *g* for 5 min at 4 °C.

Hint: Add the inhibitors at harvest, not to the culture; in living cells they shift the marks they are meant to preserve.

Hint: Mitosis-specific marks, such as phosphorylation of H3 serine 28 or H3.3 serine 31, are near-absent in asynchronous cultures. Arrest cells in G_2/M with nocodazole 🗎 SOP 0028-B, shake-off into ice-cold harvest buffer, and process alongside an asynchronous plate. Wash the pellet, not the dish; a wash over the monolayer removes the mitotic cells.

Hint: The pellet can be snap-frozen in liquid nitrogen and stored at −80 °C. Split into per-immunoprecipitation aliquots before freezing. Thaw by adding ice-cold buffer with inhibitors, never on the bench. Frozen cells lyse more readily: shorten or omit the detergent step of the nuclei isolation and check under the microscope before digesting.

(3.) Isolate nuclei from the cell pellet by detergent lysis in isosmotic sucrose 🗎 SOP 0001-B. Keep the nuclei on ice and proceed the same day.

A › *Solubilization with micrococcal nuclease*

☐ 2 × Digest adjustment buffer, pH 7.5 (200 µL / 1 rxn) ⟋ R0236 ☐ 2 × Release adjustment buffer, pH 7.5 (200 µL / 1 rxn) ⟋ R0240

(1.) Solubilize mononucleosomes by native nucleosome release 🗎 SOP 0025-A with four changes:

- Resuspend the nuclei at 5.0×10^7/mL in TNK buffer (200 µL per 1.0×10^7 cells).

  This is why: Bead capture depends on particle concentration, hence the five fold higher density (Khan et al., 2020).

- Omit the glycerol from the digestion buffer.
- Dose the nuclease at 0.5 U per 1.0×10^6 cells (25 U/mL at this density).
- Stop the digestion by chelation with EGTA, then release in 1 vol Tris/EDTA. Do not proceed with salt extraction! Set aside a tenth of each fraction for the digestion check.

  This is why: RIPA lysis with DNase I digest fails for core histones: the SDS, deoxycholate, and EDTA in RIPA inhibit DNase I, so histones stay chromatinized and are discarded with the pellet of the clearing spin. Native nucleosome release recovers about 75% of the total histone as mononucleosomes (Khan et al., 2020).

(2.) Adjust fractions S2 and S3 (about 180 µL per 1.0×10^7 cells each) separately. Add an equal volume of ice-cold 2 × buffer dropwise while vortexing at low speed:

- To S2, add digest adjustment buffer.
- To S3, add release adjustment buffer.

Critical: Avoid foaming or vigorous mixing. Abrupt salt changes can strip nucleosome-associated proteins.

(3.) Pool the two adjusted fractions (about 720 µL per 1.0×10^7 cells), clear at 13 000 × *g* for 5 min at 4 °C, and transfer the supernatant to a fresh tube on ice. ⏲ 5 min

B › *Solubilization with a universal nuclease*

- ☐ Nucleosome IP buffer, pH 7.5 (800 µL / 1 rxn) ◇ R0237
- ☐ Universal nuclease, salt-tolerant endonuclease

(1.) Resuspend the nuclear pellet in 800 µL per 1.0×10^7 cells (1.25×10^7/mL) of nucleosome IP buffer with fresh inhibitors containing universal nuclease at 25 U/mL.

Hint: Extend the incubation if the lysate stays viscous.

(2.) Incubate for 30–60 min at 4 °C with end-over-end rotation. ⏲ 60 min

(3.) Clear at 13 000 × *g* for 5 min at 4 °C and transfer the supernatant to a fresh tube. ⏲ 5 min

(4.) Set aside a tenth of the lysate for the digestion check. The remainder, about 720 µL per 1.0×10^7 cells, is already at capture conditions.

Quality assurance: A complete digest is no longer viscous.

›› *Capture*

- ☐ Nucleosome IP buffer, pH 7.5 (500 µL / 1 rxn) ◇ R0237
- ☐ Capture resin, agarose bead slurry (25 µL / 1 rxn)

(1.) *Critical:* Set aside 30 µL of the cleared chromatin as input and store it at 4 °C until the eluates are ready.

Hint: This is about 4% of the material for one IP.

(2.) Equilibrate 25 µL of capture resin slurry per immunoprecipitation, or the antibody-loaded Protein A/G beads, in 500 µL ice-cold IP buffer. Pellet the beads at 2 500 × *g* for 5 min at 4 °C and discard the supernatant. Prepare the beads for the control capture alongside. ⏲ 5 min

Hint: Below about 5.0×10^6 cells, use 10 µL slurry and elute in 20 µL.

Critical: Cut the pipette tip or use a wide-bore tip to transfer the slurry. Resuspend beads by adding buffer and inverting, never by vortexing, and do not let them dry.

(3.) Add the cleared lysate to the equilibrated beads and rotate end-over-end overnight at 4 °C. ⏲ 12 h

Hint: Vendors typically specify 1 h as the minimum for soluble proteins; nucleosomes diffuse slowly and may expose the epitope only intermittently, hence capture overnight.

(4.) Pellet the beads at 2 500 × *g* for 5 min at 4 °C and store the supernatant as flow-through with the input. ⏲ 5 min

›› *Washing and elution*

- ☐ Nucleosome IP buffer, pH 7.5 (2.5 mL / 1 rxn) ◇ R0237
- ☐ High-stringency wash buffer, pH 7.5 (1 mL / 1 rxn, *optional*) ◇ R0238
- ☐ 10% Triton™ X-100 ◇ R0057
- ☐ Elution buffer (40 µL / 1 rxn) (◇ R0104 for 4 ×)

(1.) Wash the beads three times with 500 µL ice-cold IP buffer: add the buffer, invert the tube several times, pellet at 2 500 × *g* for 5 min at 4 °C, and aspirate the supernatant. ⏲ 20 min

Critical: Do not vortex or pipette the beads up and down. Leave a small cushion of buffer over the beads between washes.

(2.) Wash twice more with 500 µL ice-cold IP buffer supplemented with Triton X-100 to 0.5%. During the last wash, transfer the bead suspension to a fresh tube before pelleting. 15 min

This is why: The tube change leaves behind protein adsorbed to the tube wall during the overnight incubation.

Hint: Background is washed out with detergent at constant salt (Khan et al., 2020); above 400 mM NaCl the linker histone is mobilized and chromatin-binding protein interactions can be disrupted SOP 0025.

(3.) *Optional:* If background persists on a nanobody or antibody-coupled resin, replace the second wash series with two washes of 500 µL high-stringency wash buffer.

Hint: Nanobody resins tolerate NaCl up to 1 M and non-ionic detergent up to 2%, but no SDS; antibody-loaded beads follow the limits of the soluble-protein protocol SOP 0027. These are resin limits, not particle limits: raise the salt only for a tag readout, not when nucleosome-associated proteins are read.

(4.) Remove the residual buffer and, depending on the readout, elute the beads:

- For immunoblotting, resuspend the beads in 40 µL 2 × Laemmli sample buffer and heat at 95 °C for 5 min. Pellet the beads at 2 500 × *g* for 2 min and transfer the supernatant to a fresh tube. 10 min
- For native elution, elute under acidic conditions SOP 0027-B for mass spectrometry or with the competitor peptide SOP 0027-C.
- For DNA sequencing, elute in 500 µL of 50 mM Tris-Cl pH 7.5, 50 mM NaCl, 5 mM EDTA, 1% SDS for 30 min at room temperature with rotation, then purify the DNA from the supernatant and from the input SOP 0024.

Hint: Enrichment is called against the digestion pattern of the input DNA.

Resource: For sequencing, follow the ENCODE histone ChIP-seq standards (https://www.encodeproject.org/): at least two biological replicates, an input library of matching run type and read length for each, and per replicate 2.0×10^7 usable fragments for narrow marks such as H3K27ac or 4.5×10^7 for broad marks such as H3K27me3 and H3K36me3.

Analyses

- Check the digestion on the set-aside aliquots SOP 0025.

Quality assurance: After MNase, a mononucleosome band at about 150 bp with a minor dinucleosome band; after the universal nuclease, a low-molecular-weight smear, which does not prove mononucleosomes.

- Resolve input, flow-through, eluate, and control eluate by SDS-PAGE SOP 0007 on a gel that resolves the tag shift, blot SOP 0008, and immunostain SOP 0009 for the epitope tag or the targeted variant or modification and for total histone H3. Bring the 30 µL input aliquot and an equal aliquot of the flow-through to 1 × Laemmli sample buffer, and load 10 µL of each alongside 10 µL of the eluate.

Hint: A 1.4 kDa V5 tag separates from endogenous H3 on a 15% gel run until the 10 kDa marker nears the bottom.

Hint: The input and flow-through lanes hold about 1% of the loaded chromatin, the eluate lane a quarter of the immunoprecipitation. Read depletion between input and flow-through.

Quality assurance: The captured histone must be depleted from the flow-through by at least half, and total H3 must be absent from the control eluate. With a tagged bait, total H3 in the eluate runs as a doublet of tagged and endogenous H3 at about equal intensity; an excess of the tagged band indicates free histone or dissociated particles. After the universal nuclease, the doublet shows intact particles but not their size. With a modification-specific antibody, the modification must be enriched over total H3 in the eluate relative to the input.

- With a tagged bait, score modifications on the shifted band, normalized to its tag or total H3 signal.

This is why: The lower band is the co-precipitated endogenous H3 of the same nucleosome; it controls for *cis* effects and does not report on the construct.

Critical: After a modification-specific IP, a co-occurring mark cannot be assigned to either H3 copy of the nucleosome.

○ *Optional:* For DNA sequencing, run the eluted and the input DNA on an agarose gel SOP 0032 before library preparation and record the yield per 1.0×10^7 cells.

> ***Quality assurance:*** The eluted DNA must show the mononucleosome band of the input without a low-molecular-weight smear; the control must yield no visible band.

Reporting standards

Parameter	E/D	Description	Standard
Input material	E	◦ Cell line, construct and tag, cell number per IP; inhibitor supplement ⋆ Cell-cycle state or arrest, passage number, and induction conditions	
Chromatin solubilization	E	◦ Nuclease, units per cell number, time and temperature, and fractions pooled ⋆ Digestion-check gel image with fragment size	
Capture reagent	E	◦ Resin or antibody with vendor, catalog number, and amount per IP ⋆ RRID, lot number, and antibody characterization data	ENCODE
Binding, washes, and elution	E	◦ IP buffer, incubation time, wash series, and elution mode ⋆ Control IP performed and fraction of eluate loaded	
Readout	E	◦ Detection antibodies, the band scored (tagged or endogenous), and the input, flow-through, and control lanes shown ⋆ Normalization to tag or total histone signal; for sequencing, replicate number, input library, and usable fragments per replicate	ENCODE

Document the essential (E) and desirable (D) parameters where feasible to meet the reproducibility standards expected by journals and data/research repositories — at the minimal (◦) or extended (⋆) level.

Troubleshooting

Solubilization with micrococcal nuclease

In Step 1:

– The digest stays viscous, or the chelator release yields little material
  - ◦ Confirm that no chelator reached the digestion; protease-inhibitor tablets often carry EDTA.
  - ◦ Extend the digestion rather than raising the nuclease; over-digestion cuts into the nucleosome core and loses the fragments below 150 bp.
  - ◦ Check the nuclei: clumping on resuspension means lysed nuclei and released genomic DNA, not an incomplete digest SOP 0001-B.

Capture

In Step 3:

– The captured histone is not depleted from the flow-through
  - ◦ Resolve the pellet of the clearing spin alongside the input; histones left there point to the digestion, not the capture.
  - ◦ Confirm that the tag is accessible on the assembled nucleosome. A tag that captures the free histone can be occluded once the particle is built.
  - ◦ Keep the volumes as given; diluting the chromatin costs yield.

Washing and elution

In Step 2:

– Histone or reader signal in the control eluate
  - ◦ Add wash cycles at 0.5% Triton X-100 before raising the salt; the particle survives detergent.
  - ◦ Pre-clear the adjusted chromatin on uncoupled beads of the same matrix before capture SOP 0027.
  - ◦ Prepare the control in parallel; a parental lysate grown, harvested, digested, or adjusted on another day scores handling, not specificity.

Materials

Harvest and isolation of nuclei

- ☐ **Harvest buffer**
  Hint: Supplement the buffer with the inhibitor supplement on the day of use.
  - **Phosphate-buffered saline (PBS)**, pH 7.5 (at 1×) ◇ R0037
- ☐ **Inhibitor supplement**
  Critical: Add the supplement fresh to every buffer, from harvest through to the last wash. ***This is why:*** The supplement carries phosphatase, deacetylase, and serine-protease inhibitors.
  - **Calyculin A**, 100 µM ◇ R0239
    Store at −20 °C.
  - **β-Glycerophosphate (BGP)**, 1 M ◇ R0164
  - **Trichostatin A (TSA)**, 20 mM ◇ R0165
  - **Nicotinamide (NAM)**, 1 M ◇ R0166
  - **Phenylmethylsulfonyl fluoride (PMSF)**, 100 mM ◇ R0035

Solubilization with micrococcal nuclease

- ☐ **Digest adjustment buffer**, 2×, pH 7.5 ◇ R0236 (200 µL / 1 rxn)
  Store at 4 °C.
- ☐ **Release adjustment buffer**, 2×, pH 7.5 ◇ R0240 (200 µL / 1 rxn)
  Store at 4 °C.

Solubilization with a universal nuclease

- ☐ **Nucleosome IP buffer**, pH 7.5 ◇ R0237 (800 µL / 1 rxn)
- ☐ **Universal nuclease**, salt-tolerant endonuclease
  This is why: The nuclease cuts both DNA and RNA, and stays active in the 2 mM $MgCl_2$ of this buffer.
  - **Benzonase® Nuclease**

Capture

- ☐ **Nucleosome IP buffer**, pH 7.5 ◇ R0237 (500 µL / 1 rxn)
  Store at 4 °C.
- ☐ **Capture resin** (25 µL / 1 rxn), agarose bead slurry
  Hint: For antibody capture: Protein A/G beads, loaded with the antibody beforehand and, if desired, crosslinked to it.
  - *e.g.* **V5-Trap® Agarose**
    ChromoTek, v5ta, RRID AB_2868497
  - *e.g.* **GFP-Trap® Agarose**
    ChromoTek, gta, RRID AB_2631357
  - *e.g.* **Spot-Trap® Agarose**
    ChromoTek, eta, RRID AB_2827590
  - *e.g.* **Anti-Flag® M2 Affinity Gel**
    Millipore®, A2220, RRID AB_10063035
  - *e.g.* **Protein A/G beads**, antibody-loaded

Washing and elution

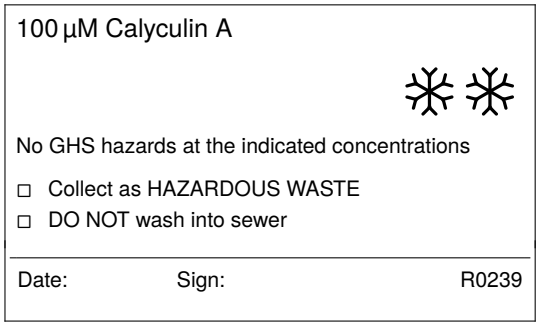
- ☐ **Nucleosome IP buffer**, pH 7.5 ◇ R0237 (2.5 mL / 1 rxn)
- ☐ **Triton™ X-100**, 10% ◇ R0057
  Hint: This raises the detergent of the IP buffer for the second wash series.
- ☐ **High-stringency wash buffer**, pH 7.5 ◇ R0238 (1 mL / 1 rxn, *optional*)
  Store at 4 °C.
- ☐ **Elution buffer** (40 µL / 1 rxn)
  - **Laemmli sample buffer**, pH 6.8 ◇ R0104

Recipes

Calyculin A, 100 µM

Amount	Ingredient		Stock	Final
100 µg	Calyculin A	[101932-71-2]	1 009.2 g/mol	100 µM
To 1 mL	Dimethyl sulfoxide (DMSO), reagent-grade			

Dissolve the content of a 100 µg vial in DMSO and dispense into 20 µL aliquots. Store at −20 °C. ***Critical:*** Calyculin A is cell-permeable and acutely toxic; handle powder and stock with gloves. ***This is why:*** A PP1 and PP2A inhibitor; PP1 is the phosphatase that balances H3 serine phosphorylation through mitosis [HSL+00].



Digest adjustment buffer, pH 7.5, 2 ×

Amount	Ingredient		Stock	Final
300 µL	HEPES, pH 7.5	R0024	1 M	30 mM
450 µL	Sodium chloride (NaCl)	R0046	5 M	225 mM
30 µL	Magnesium chloride ($MgCl_2$)	R0031	1 M	3 mM
400 µL	Triton™ X-100	R0057	10%	0.4%
	Skin irritation (H315); Serious eye damage (H318); Aquatic toxicity (long-term) (H411)			
4 mL	Glycerol	R0022	50%	20%
200 µL	Inhibitor cocktail	☐ R0090	100 ×	2 ×
	Flammable liquid (H225); Serious eye irritation (H319); Reproductive toxicity (H361)			
To 10 mL	Water, reagent-grade			

Add inhibitor cocktail immediately before use. Store at 4 °C. ***Note:*** For fraction S2 only; buffer E of the source protocol (Khan et al., 2020).

2 × Digest adjustment buffer

15 mM HEPES, 113 mM NaCl, 1.5 mM $MgCl_2$,
0.2% Triton™ X-100, 10% Glycerol,
☐ 1 × Inhibitor cocktail, pH 7.5 *[At 1 × dilution]*



WARNING



Reproductive toxicity; Aquatic toxicity (long-term)

☐ Collect as HAZARDOUS WASTE
☐ DO NOT wash into sewer

Date: Sign: R0236

Release adjustment buffer, pH 7.5, 2 ×

Amount	Ingredient		Stock	Final
300 µL	HEPES, pH 7.5	R0024	1 M	30 mM
600 µL	Sodium chloride (NaCl)	R0046	5 M	300 mM
30 µL	Magnesium chloride ($MgCl_2$)	R0031	1 M	3 mM
400 µL	Triton™ X-100	R0057	10%	0.4%
	Skin irritation (H315); Serious eye damage (H318); Aquatic toxicity (long-term) (H411)			
4 mL	Glycerol	R0022	50%	20%
200 µL	Inhibitor cocktail	☐ R0090	100 ×	2 ×
	Flammable liquid (H225); Serious eye irritation (H319); Reproductive toxicity (H361)			
To 10 mL	Water, reagent-grade			

Add inhibitor cocktail immediately before use. Store at 4 °C. ***Note:*** For fraction S3 only; the 2 × form of the nucleosome IP buffer, which the source protocol uses as a 3 × concentrate, buffer D (Khan et al., 2020).

2 × Release adjustment buffer

15 mM HEPES, 150 mM NaCl, 1.5 mM $MgCl_2$,
0.2% Triton™ X-100, 10% Glycerol,
☐ 1 × Inhibitor cocktail, pH 7.5 *[At 1 × dilution]*



WARNING



Reproductive toxicity; Aquatic toxicity (long-term)

☐ Collect as HAZARDOUS WASTE
☐ DO NOT wash into sewer

Date: Sign: R0240

Nucleosome IP buffer, pH 7.5

Amount	Ingredient		Stock	Final
750 µL	HEPES, pH 7.5	R0024	1 M	15 mM
1.5 mL	Sodium chloride (NaCl)	R0046	5 M	150 mM
75 µL	Magnesium chloride ($MgCl_2$)	R0031	1 M	1.5 mM
1 mL	Triton™ X-100	R0057	10%	0.2%
	Skin irritation (H315); Serious eye damage (H318); Aquatic toxicity (long-term) (H411)			
10 mL	Glycerol	R0022	50%	10%
500 µL	Inhibitor cocktail	☐ R0090	100 ×	1 ×
	Flammable liquid (H225); Serious eye irritation (H319); Reproductive toxicity (H361)			
To 50 mL	Water, reagent-grade			

Add inhibitor cocktail immediately before use. Store at 4 °C. ***Critical:*** Replace HEPES with Tris-Cl only when no mass spectrometry is planned; never add SDS. ***Hint:*** The general IP buffer of the soluble-protein protocol SOP 0027, made chelator-free and buffered with HEPES. Physiological salt holds the particle together and suppresses electrostatic sticking to the beads, magnesium keeps the particle compact and a universal nuclease active, and the detergent lowers hydrophobic background.

Nucleosome IP buffer

15 mM HEPES, 150 mM NaCl, 1.5 mM $MgCl_2$,
0.2% Triton™ X-100, 10% Glycerol,
☐ 1 × Inhibitor cocktail, pH 7.5



WARNING



Reproductive toxicity

☐ Collect as HAZARDOUS WASTE
☐ DO NOT wash into sewer

Date: Sign: R0237

High-stringency wash buffer, pH 7.5

Amount	Ingredient		Stock	Final
750 µL	HEPES, pH 7.5	R0024	1 M	15 mM
3 mL	Sodium chloride (NaCl)	R0046	5 M	300 mM
75 µL	Magnesium chloride ($MgCl_2$)	R0031	1 M	1.5 mM
25 µL	IGEPAL® CA-630	[9002-93-1]	100%	0.05%
10 mL	Glycerol	R0022	50%	10%
500 µL	Inhibitor cocktail	☐ R0090	100 ×	1 ×
	Flammable liquid (H225); Serious eye irritation (H319); Reproductive toxicity (H361)			
To 50 mL	Water, reagent-grade			

Add inhibitor cocktail immediately before use. Store at 4 °C. ***Note:*** IP buffer with the salt raised to 300 mM and IGEPAL CA-630 in place of Triton X-100.

High-stringency wash buffer

15 mM HEPES, 300 mM NaCl, 1.5 mM $MgCl_2$, 0.05% IGEPAL® CA-630, 10% Glycerol, ☐ 1 × Inhibitor cocktail, pH 7.5



WARNING



Reproductive toxicity

☐ Collect as HAZARDOUS WASTE
☐ DO NOT wash into sewer

Date: Sign: R0238

List of references

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Change log

2026-09-07 **Benjamin C. Buchmuller** Version 1, 2026. Bead handling and elution from the ChromoTek "V5-Trap Agarose" manual (2021); native chromatin preparation adapted from Umlauf et al., Epigenome NoE protocol "Chromatin immunoprecipitation on native chromatin from cells and tissues", and Khan et al. (2020).

2026-11-01 **Benjamin C. Buchmuller** Version 2. The nucleosome survives above 400 mM NaCl: the salt can disrupt the linker histone and chromatin-binding proteins, not the core particle. Digestion is stopped with EGTA and released in Tris/EDTA. Credits: Tsukada et al. (2006) and Shi et al. (2004) for the JmjC and LSD1 families, Hsu et al. (2000) for the aurora kinase and PP1 balance, Abcam (2026) for the fragment lengths and the rearrangement caveat.

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