

Immunoprecipitation of soluble proteins

Immunoprecipitation (IP) is a technique to isolate specific proteins or protein complexes from a biological sample using an affinity-based capture reagent, typically an antibody or affinity resin.

This protocol covers IP of native and epitope-tagged proteins from whole cell lysates, cytosolic fractions, or nuclear extracts, that is, from targets that are in solution once the cell is lysed. It is compatible with workflows for co-immunoprecipitation (co-IP) and interaction proteomics. Histones, nucleosomes, and other chromatin-bound targets follow the native chromatin protocol [SOP0047](#).

Eluted proteins may be analyzed by immunoblotting [SOP0009](#), silver staining [SOP0011](#), or mass spectrometry. For RNA- or DNA-associated proteins, crosslinking or nuclease treatment may be required to stabilize interactions or reduce non-specific background.

Risk assessment

- **Formaldehyde is a KNOWN CARCINOGEN and REPRODUCTIVE TOXIN**
- Work with human-derived material or transgenic cell lines (BSL-2)
- ▷ Perform formaldehyde crosslinking in a certified chemical fume hood
- ▷ Wear gloves, safety glasses, lab coat
- Dispose waste after inactivation as REGULATED MEDICAL WASTE



Reviewed: Sep 7, 2026

Procedures

Planning and considerations

- (1.) Choose the extraction route from the subcellular localization of the target:

Target compartment	Extraction Strategy	Protocol	Ionic strength	Adjustment	X-link possible	IP buffer
Whole cell	Strong detergent lysis	SOP0002-A	150 mM	None	✓	General IP buffer
Cytosol and Membranes	Mild detergent lysis	SOP0002-B	150 mM	None	✓	General IP buffer
Cytosol	Hypotonic lysis, nuclei removed	SOP0001-A	150 mM	None	✓	General IP buffer
Nucleosol	Low-salt nuclear extraction	SOP0025	0–75 mM	3+1 to 6+1 high-salt buffer	✓	General IP buffer
Nucleus	Total chromatin extraction	SOP0025-B	300–600 mM	1+1 to 1+3 low-salt buffer	✓	Nuclear extract IP buffer
Chromatin-associated proteins	Partial MNase digestion, salt fractionation	SOP0025-B	300–600 mM	1+1 to 1+3 low-salt buffer	✓	Nuclear extract IP buffer
Histones, acid-extracted	Acid extraction	SOP0026	No salt	Dialyze	No	Nuclear extract IP buffer
Nucleosomes	Full MNase digestion, chelator release	SOP0025-A	No salt	Not applicable	No	Not applicable

Hint: The adjustment column gives the mixing ratio in volumes for about 150 mM sodium chloride. The low-salt and high-salt buffers are described in the nuclear extraction protocol [SOP0025](#).

Hint: For immunoprecipitation of native nucleosomes, follow the native chromatin protocol [SOP0047](#).

- (2.) Decide whether to crosslink before harvest. Crosslinking is optional for soluble targets, but must not be used before acid extraction of histones [SOP0026](#).

Hint: Crosslinking fixes weak or transient interactions. It can mask epitopes and reduce solubility. Titrate concentration and time for each new target.

- (3.) Decide the capture chemistry from what is available. Choose native immunoprecipitation when a high-affinity, IP-validated antibody exists and the bait is abundant enough to be depleted from the extract with it; otherwise consider epitope pull-down.

Hint: Baits can be tagged at the endogenous locus ⓘ SOP0038, or stably ⓘ SOP0017 ⓘ SOP0018 or transiently expressed in the cell ⓘ SOP0012 ⓘ SOP0013.

Critical: Validate a tagged bait against the untagged protein; a tag or transgene expression can perturb folding, localization, and stoichiometry.

Critical: Include at least one control antibody against an irrelevant epitope such as glutathione S-transferase to check specificity.

- (4.) Choose wash stringency and elution mode from the downstream application:
- For immunoblotting or silver staining, wash in IP buffer and elute in the downstream sample buffer (*Alternative A*).
 - For mass spectrometry, keep detergent to a low concentration of NP-40 or digitonin and elute under acidic conditions (*Alternative B*).
 - For interaction studies, elute a tagged bait with competitor peptide (*Alternative C*).
- (5.) Plan the run: one day from cells to the adjusted extract, capture for hours or overnight, elution and analysis the next morning.

Hint: Apply protease inhibitor cocktail at harvest. When phosphorylation or acetylation is assessed, add phosphatase and deacetylase inhibitors to the harvest buffer, not to the culture.

⊕ **Optional: Crosslinking with formaldehyde**

- | | |
|---|---|
| ☐ 1% Formaldehyde ⓘ R0189
☐ 1.25 M Glycine, pH 7.0 ⓘ R0190 | ☐ 0.5 M Ammonium bicarbonate, pH 8.0 ⓘ R0191 |
|---|---|

- (1.) Choose the crosslinker:

Crosslinker	Reversibility	Spacer	Notes
Formaldehyde	Cleavable by heat or alkali	2.3–2.7 Å	Most common; preserves chromatin context
DSP / DTSSP	Cleavable by reducing agents	12 Å	Compatible with membrane proteins
DSS / BS3	Irreversible	11 Å	Used in structural proteomics
Glutaraldehyde	Irreversible	5 Å	Strong crosslinker; often excessive for IP; alters antigenicity

- (2.) Wash the cells once with PBS. Remove PBS completely.

Hint: Crosslink adherent cells on the dish to preserve native interactions.

- (3.) Add fresh 1% formaldehyde in PBS. Incubate for 10 min at room temperature with gentle rocking.

⌚ 10 min

Critical: Titrate formaldehyde (0.5–5%) and time (5–20 min) for the cell type and target; for most targets, do not exceed 1% or 10 min. Over-crosslinking reduces antigenicity and solubility.

- (4.) Quench the reaction by adding 0.1 vol ice-cold 1.25 M glycine (or 0.5 M ammonium bicarbonate when planning mass spectrometry). Incubate for 5–15 min at room temperature with gentle agitation.

⌚ 10 min

This is why: Ammonium bicarbonate yields a volatile product that does not interfere with mass spectrometry. For TMT or iTRAQ, quench with triethylammonium bicarbonate instead.

- (5.) Remove the quenched crosslinking solution and wash the cells twice with ice-cold PBS, then harvest and extract following the protocol chosen in the planning table.

>> Buffer adjustment for immunoprecipitation

- ☐ General IP buffer  R0156
 – or: Nuclear extract IP buffer  R0157

- (1.) Adjust the extract to the IP conditions given in the planning table (150 mM sodium chloride, 10–20% glycerol, 0.1% mild detergent, pH 7.4). If the upstream buffer carries no glycerol, supplement it during the dilution or the dialysis. Add diluents slowly while vortexing at low speed. 

Critical: Avoid foaming or vigorous mixing, which denatures and aggregates protein complexes. 

- (2.) *Optional:* For acid-extracted histones, high-salt nuclear fractions, or mass spectrometry, dialyze against 1 × IP buffer overnight. 

- (3.) Clear the sample at 13 000 × g for 5 min at 4 °C. Transfer the supernatant to a fresh tube.

> Preparation of antibody-coated beads

- ☐ Protein A/G beads, 50 percent slurry

- (1.) Transfer 20–50 µL Protein A or Protein G slurry per immunoprecipitation to a low-binding tube. Prepare one tube for the sample and one for the negative control. 

Hint: Protein A binds best to rabbit and some human IgG subclasses; Protein G binds a broader range of IgGs including mouse. Use a mix (A/G) when uncertain.

- (2.) Wash the beads three times with 0.5 mL cold IP buffer. Pellet on a magnetic stand or at 700 × g for 15 s at 4 °C. Remove the supernatant and resuspend at once. Do not let beads dry.

- (3.) *Critical:* Coat the sample beads with 0.5–5.0 µg antibody in 200 µL IP buffer. For the negative control, coat beads with the same amount of a non-specific IgG of the same species and isotype. Rotate end-over-end for 30–60 min at 4 °C.   1 h 

Quality assurance: Titrate a new antibody once: three amounts on constant lysate, scored as depletion of the bait from the flow-through. Use the smallest amount that depletes it. 

Resource: Use IP-validated antibodies; the ENCODE antibody registry (<https://www.encodeproject.org/antibodies/>) lists characterized lots for chromatin-associated proteins. 

- (4.) Wash the beads twice with 0.5 mL cold IP buffer.

- (5.) *Optional:* Crosslink the antibody to the beads by resuspending them in 200 µL freshly prepared 20 mM dimethyl pimelimidate (DMP) in 200 mM triethanolamine buffer, pH 8.9, for 30 min at room temperature. Quench by adding 50 µL of 1 M Tris, pH 7.5, incubate for 15 min. Wash as before. 

This is why: Crosslinking the antibody to the beads keeps the heavy and light chains out of the eluate.

> Preparation of affinity resin for tagged baits

- ☐ Affinity resin for tagged proteins, pre-conjugated

- (1.) Transfer enough pre-conjugated resin slurry to capture 5–10 µg of the tagged protein, typically 10–30 µL per IP, to a low-binding tube with a wide-bore tip. Prepare one tube for the sample and one for the negative control. 

- (2.) Wash the resin three times with 0.5 mL cold IP buffer. Pellet on a magnetic stand or at 700 × g for 15 s at 4 °C. Remove the supernatant and resuspend at once. Do not let beads dry.

⊕ Optional: Pre-clearing lysates

- (1.) Prepare a bead slurry for pre-clearing. Wash 20–50 μL of Protein A/G agarose or magnetic beads in immunoprecipitation buffer and resuspend as a 50% slurry.

This is why: Pre-clearing removes proteins that bind non-specifically to beads; omit it when background is known to be low or with non-porous beads such as Dynabeads™.

Critical: Use the same bead type as in the final IP. Do not reuse these beads for the actual IP.

- (2.) Add washed beads to the extract. Incubate at 4 °C for 30–60 min with gentle rotation.
- (3.) Pellet the beads (centrifuge or magnet) and transfer the pre-cleared supernatant to a fresh tube.
- (4.) *Optional:* Retain the bead pellet for analysis if bait loss is suspected.



⌚ 1 h

**» Capture**

- (1.) *Critical:* Save 5–10% of the lysate as input and store it at 4 °C until the eluates are ready.
- (2.) Add 0.5–2.0 mL pre-cleared lysate (containing 100–500 μg total protein) to the beads. Prepare the negative control by adding the same lysate to the isotype control beads, or lysate from cells that do not express the tagged bait to the epitope pull-down.



- (3.) Rotate end-over-end at 4 °C for 30–120 min for an epitope pull-down, or 1–4 h or overnight for a native IP or a weakly expressed tagged bait.

⌚ 2 h

Hint: High-efficiency tags like FLAG, HA, Strep, or His also bind within 20–30 min at room temperature, which sometimes improves recovery of weak interactors at the cost of background and degradation.

- (4.) *Critical:* Pellet the beads as before and keep the supernatant as flow-through. Store it with the input.
- (5.) Wash the beads three times with 0.5 mL cold IP buffer. Wash up to five times for an epitope pull-down. Transfer the beads to a fresh tube in the last wash.



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

Hint: For higher stringency, raise sodium chloride to 300–500 mM or add up to 0.5% Triton™ X-100 or NP-40; pre-conjugated resins tolerate this better than antibody-loaded Protein A/G beads. For native co-immunoprecipitation, avoid high salt and ionic detergents such as SDS.

A > Elution in sample buffer

☐ 2 × Laemmli sample buffer (⚡ R0104 for 4 ×)

- (1.) Remove the residual buffer, resuspend the beads in 80 μL 2 × Laemmli sample buffer, and heat at 95 °C for 5 min. Pellet the beads and transfer the supernatant to a fresh tube.

⌚ 10 min

Hint: The default for immunoblotting and silver staining.

B > Elution under acidic conditions

☐ Acidic elution buffer, pH 2.5 ⚡ R0160

☐ Neutralization base, pH 10.4 ⚡ R0161

- (1.) Add 50–100 μL acidic elution buffer. This is 1.0 vol. Pipette continuously for 30–60 s to mix.

Hint: Elution at room temperature is more efficient than on ice.

Critical: Desalt before mass spectrometry.



- (2.) Pellet the beads as before. Transfer the clear supernatant to a new tube.
- (3.) Immediately neutralize with 0.1 vol neutralization buffer to prevent hydrolysis.
- (4.) Repeat the elution at least once to improve recovery. Pool fractions as needed.

C > **Elution with competitor peptide**

□ 1 × Phosphate-buffered saline, pH 7.4 (↗ R0037 for 10 ×)

- (1.) Reconstitute the competitor peptide in PBS to a final concentration of 1–2 mM or 1.5–3.0 g/L.
- (2.) Dilute the peptide stock to 0.5 mM in PBS.
- (3.) Add 100 µL of the diluted peptide. Incubate for 10–20 min at room temperature or for 60 min at 4 °C. ⌚ 20 min

Hint: Peptide elution preserves folding for interaction studies. For mass spectrometry, prefer acidic elution; the peptide adds background.

- (4.) Pellet the beads as before. Transfer the clear supernatant to a new tube.
- (5.) Repeat the elution at least once to increase recovery.

Analyses

- Load input, flow-through, eluate, and control eluate side by side, the same fraction of each, and resolve by SDS-PAGE ↗ SOP0007 followed by immunoblotting ↗ SOP0009 or silver staining ↗ SOP0011. Centrifuge the samples briefly to clear before loading.

Hint: Load the equivalent of 1.0×10^6 – 2.5×10^6 cells, or 1–2 µg input material, per IP.

- Score the capture. Immunoblot for the bait, or its tag, and for the partners or modifications of interest.

Quality assurance: The bait must be depleted from the flow-through by at least half relative to the input and absent from the control eluate. Normalize co-precipitated partners to the bait in the eluate. ⚡

- *Optional:* For mass spectrometry, submit at least three biological replicates of the bait and its control, captured on the same bead lot and processed together. ⊕

Reporting standards

Parameter	E/D	Description	Standard
Input material	E	○ Cell line, cell number per IP, and upstream extraction protocol ★ Crosslinking conditions, passage number, and treatment	
Capture reagent	E	○ Antibody or resin with vendor, catalog number, and amount per IP ★ RRID, lot number, and characterization data	ENCODE
Binding and washes	E	○ IP buffer, incubation time and temperature, number and composition of washes ★ Pre-clearing and control IP performed	
Elution	E	○ Elution mode and volume ★ Fraction of eluate loaded per lane or submitted	
Readout	E	○ Detection antibodies and the input, flow-through, and control lanes shown ★ Quantification method and normalization	

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.

Materials

Crosslinking with formaldehyde

Formaldehyde ⚗ R0189, 1%, Stable for 4 weeks at room temperature.

Glycine ⚗ R0190, 1.25 M, pH 7.0, Stable for 2 weeks at 4 °C.

Ammonium bicarbonate ⚗ R0191, 0.5 M, pH 8.0, Stable for 1 week at 4 °C.

Buffer adjustment for immunoprecipitation

IP buffer

- **General IP buffer** ⚗ R0156, Store at room temperature.
- **or Nuclear extract IP buffer** ⚗ R0157, Store at room temperature.

Preparation of antibody-coated beads

Protein A/G beads, 50 percent slurry

Preparation of affinity resin for tagged baits

Affinity resin for tagged proteins, pre-conjugated

Elution in sample buffer

2 × Laemmli sample buffer (⚗ R0104 for 4 ×)

Elution under acidic conditions

Acidic elution buffer ⚗ R0160, pH 2.5

Neutralization base ⚗ R0161, pH 10.4

Elution with competitor peptide

1 × Phosphate-buffered saline (⚗ R0037 for 10 ×), pH 7.4

Recipes

Formaldehyde (FA), 1%

Amount	Ingredient	Stock	Final
1 mL	Phosphate-buffered saline (PBS), pH 7.4 ⚗ R0037	10 ×	1 ×
270 µL	Formaldehyde, in water, stabilized with methanol [50-00-0] Skin corrosion (H314); Mutagenicity (H341); Carcinogenicity (H350)	37%	1%
To 10 mL	Water, reagent-grade		

Prepare fresh before use. Stable for 4 weeks at room temperature. **Hint:** When starting from paraformaldehyde, bring to 80 °C for 1–2 h and filter through a 0.22 µm pore.

1% Formaldehyde (FA)

1 × PBS, 1% Formaldehyde



DANGER

Carcinogenicity; Skin sensitization; Germ cell mutagenicity

- ☐ Collect as HAZARDOUS WASTE
- ☐ DO NOT wash into sewer

Date: Sign: R0189

Glycine, pH 7.0, 1.25 M

Amount	Ingredient	Stock	Final
4.69 g	Glycine [56-40-6]	75.07 g/mol	1.25 M
To 50 mL	Water, reagent-grade		

Adjust pH with hydrochloric acid. Stable for 2 weeks at 4 °C.

1.25 M Glycine

pH 7.0



EPA Safer Choice low-concern

- ☐ Flush to drain with excess water

Date: Sign: R0190

Ammonium bicarbonate, pH 8.0, 0.5 M

Amount	Ingredient		Stock	Final
1.98 g	Ammonium bicarbonate	[1066-33-7]	79.06 g/mol	0.5 M
To 50 mL	Water, reagent-grade			

Prepare fresh before use. Stable for 1 week at 4 °C.

**0.5 M Ammonium bicarbonate
pH 8.0**

No GHS hazards at the indicated concentrations

☐ Hold for EHS review before disposal

Date: Sign: R0191

General IP buffer

Amount	Ingredient		Stock	Final
0.5 mL	Tris-Cl, pH 7.4	⚡ R0055	1 M	10 mM
1.5 mL	Sodium chloride (NaCl)	⚡ R0046	5 M	150 mM
10 mL	Glycerol	⚡ R0022	50%	10%
100 µL	MgCl ₂	⚡ R0031	1 M	2 mM
200 µL	EGTA, pH 8.0	⚡ R0145	0.5 M	2 mM
500 µL	Triton™ X-100	⚡ R0057	10%	0.1%
Skin irritation (H315); Serious eye damage (H318); Aquatic toxicity (long-term) (H411)				
50 µL	Dithiothreitol (DTT)	☐ ⚡ R0015	1 M	1 mM
Skin irritation (H315); Serious eye irritation (H319)				
500 µL	Inhibitor cocktail	☐ ⚡ R0090	100 ×	1 ×
Flammable liquid (H225); Serious eye irritation (H319); Reproductive toxicity (H361)				

To 50 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:** For cytosolic proteins and soluble nuclear factors. Triton™ X-100 can be replaced with another non-ionic detergent such as IGEPAL® CA-630, the substitute for the discontinued NP-40.

Nuclear extract IP buffer

Amount	Ingredient		Stock	Final
1.0 mL	Tris-Cl, pH 7.4	⚡ R0055	1 M	20 mM
1.7 mL	Potassium chloride (KCl)	⚡ R0038	3 M	100 mM
20 mL	Glycerol	⚡ R0022	50%	20%
20 µL	EDTA, pH 8.0	⚡ R0017	0.5 M	0.2 mM
50 µL	Triton™ X-100	⚡ R0057	10%	0.01%
Skin irritation (H315); Serious eye damage (H318); Aquatic toxicity (long-term) (H411)				
50 µL	Dithiothreitol (DTT)	☐ ⚡ R0015	1 M	1 mM
Skin irritation (H315); Serious eye irritation (H319)				
500 µL	Inhibitor cocktail	☐ ⚡ R0090	100 ×	1 ×
Flammable liquid (H225); Serious eye irritation (H319); Reproductive toxicity (H361)				

To 50 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:** Dignam buffer D, the dialysis buffer for salt-extracted nuclear proteins; heavy glycerol and minimal detergent hold fragile complexes together. For high-salt nuclear extracts and acid-extracted histones.

General IP buffer

10 mM Tris-Cl, 150 mM NaCl, 10% Glycerol,
2 mM MgCl₂, 2 mM EGTA, 0.1% Triton™ X-100,
☐ 1 mM DTT, ☐ 1 × Inhibitor cocktail



WARNING

Reproductive toxicity

☐ Collect as HAZARDOUS WASTE
☐ DO NOT wash into sewer

Date: Sign: R0156

Nuclear extract IP buffer

20 mM Tris-Cl, 100 mM KCl, 20% Glycerol,
0.2 mM EDTA, 0.01% Triton™ X-100,
☐ 1 mM DTT, ☐ 1 × Inhibitor cocktail



WARNING

Reproductive toxicity

☐ Collect as HAZARDOUS WASTE
☐ DO NOT wash into sewer

Date: Sign: R0157

Triethanolamine solution, pH 8.9, 200 mM

Amount	Ingredient	Stock	Final
264 µL	Triethanolamine (TEA) [102-71-6]	149.19 g/mol	200 mM
To 10 mL	Water, reagent-grade		

Adjust to pH 8.9 with hydrochloric acid or sodium hydroxide as needed.
Stable for 2 weeks at 4 °C.

200 mM Triethanolamine solution
pH 8.9



No GHS hazards at the indicated concentrations

☐ Hold for EHS review before disposal

Date: Sign: R0158

Dimethyl pimelimidate (DMP), 20 mM

Amount	Ingredient	Stock	Final
51.8 mg	Dimethyl pimelimidate · 2 HCl (DMP) [58537-94-3]	259.17 g/mol	20 mM
To 10 mL	Triethanolamine buffer, pH 8.9		

Prepare fresh immediately before use.

20 mM Dimethyl pimelimidate (DMP)

No GHS hazards at the indicated concentrations

☐ Hold for EHS review before disposal

Date: Sign: R0159

Acidic elution buffer, pH 2.5

Amount	Ingredient	Stock	Final
0.75 g	Glycine [56-40-6]	75.07 g/mol	200 mM
To 50 mL	Water, reagent-grade		

Adjust the pH to 2.5 at 4 °C.

Acidic elution buffer

pH 2.5



WARNING

Skin and serious eye irritation

☐ Neutralise to pH 6–9

☐ Flush to drain with excess water

Date: Sign: R0160

Neutralization base, pH 10.4

Amount	Ingredient	Stock	Final
6.06 g	Tris base [77-86-1]	121.14 g/mol	1 M
To 50 mL	Water, reagent-grade		

Adjust the pH to 10.4 at 4 °C.

Neutralization base

pH 10.4



WARNING

Skin and serious eye irritation

☐ Neutralise to pH 6–9

☐ Dilute below the discharge limit

☐ Flush to drain with excess water

Date: Sign: R0161

Troubleshooting

Buffer adjustment for immunoprecipitation

In Step 1:

- Protein precipitation or loss of bait protein after dilution into minimal IP buffer (Tris/NaCl/NP-40 only)
 - Include 5–10% glycerol and 1–2 mM MgCl₂ or EGTA to stabilize protein complexes and reduce aggregation.
 - Reduce dilution factor or dialyze gradually to avoid abrupt changes in ionic strength.
- High background binding or non-specific interactions
 - Add 0.01–0.1% Triton™ X-100 or IGEAL CA-630 to reduce hydrophobic interactions.
 - Increase salt concentration to 200–300 mM sodium chloride or include 0.01–0.05% Tween-20 if compatible with downstream applications.
- Loss of weak or transient interactions
 - Perform binding at 4 °C for only 30–60 min.

Capture

In Step 5:

- Strong bands at 50 kDa and 25 kDa obscure target signal
 - Possibly IgG heavy and light chains from the IP antibody: Crosslink the antibody to the beads or elute under native conditions.
 - Use a light chain- or heavy chain-specific secondary antibody for detection.
- Low IP efficiency
 - Check epitope accessibility; tags buried in a complex or masked by a partner capture poorly.
 - For His-tagged baits, avoid strong reducing agents and chelators, which interfere with the metal ion of the resin.

Resources

Planning and considerations

	Native immunoprecipitation	Epitope pull-down
Requires	IP-validated antibody against the bait	Epitope tag on the bait, pre-conjugated resin
Bead	Protein A/G, antibody loaded on the day or crosslinked	Resin coupled to antibody or nanobody
Elate	Heavy and light chain unless the antibody is crosslinked	No immunoglobulin from nanobody resins
Wash stringency	Limited by the antibody–antigen and antibody–bead bonds	High salt and non-ionic detergent tolerated
Native elution	Acidic	Acidic or competitor peptide
Negative control	Isotype IgG on the same beads	Untagged lysate on the same resin

In Step 3: What each capture chemistry needs and yields.

Preparation of antibody-coated beads

Beads	Format	Capacity	Per 10 µg	Manufacturer	Cat. No.
nProtein A Sepharose™ 4 Fast Flow	Sepharose	30 g/L	0.3 µL	Cytiva™ Life Science	17-5280-01
Protein G Sepharose™ 4 Fast Flow	Sepharose	20 g/L	0.5 µL	Cytiva™ Life Science	17-0618-01
Pierce™ Protein A/G Magnetic Beads	Magnetic	55–85 µg/mg	12–18 µL	Thermo Scientific™	88802
Dynabeads™ Protein G	Magnetic	8–10 µg/mg	33–40 µL	Invitrogen™	10003D

In Step 1: Protein A and Protein G beads with the manufacturer's binding capacity and the volume that binds 10 µg immunoglobulin (settled medium for Sepharose, supplied suspension for magnetic beads). The volumes used here far exceed the immunoglobulin loaded.

Isotype control	Clone	Manufacturer	Cat. No.	RRID
Normal Mouse IgG	Polyclonal	Cell Signaling Technology	68860	AB_3675987
Mouse IgG1	G3A1	Cell Signaling Technology	5415	AB_10829607
Normal Rabbit IgG	Polyclonal	Sino Biological	CR1	AB_3073921

In Step 3: Isotype controls.

Preparation of affinity resin for tagged baits

Epitope tag	Affinity resin	Capacity	Per 10 µg	Manufacturer	Cat. No.	RRID
Biotin	Streptavidin Sepharose™ High Performance	6.0 g/L	8 µL	Cytiva™ Life Science	17-5113-01	
FLAG tag	Anti-Flag® M2 Affinity Gel	0.6 g/L	30 µL	Millipore®	A2220	AB_10063035
	Anti-DYKDDDDK Magnetic Agarose	3.2 g/L	12 µL	Thermo Scientific™	A36797	AB_3106985
Strep tag	Strep-Tactin® XT 4Flow®	5.0 g/L	10 µL	IBA Life Science	2-5010	
V5 tag	V5-Trap® Magnetic Agarose	0.7 g/L	14 µL	ChromoTek	v5tma	AB_2868498

In Step 1: Affinity resins for common epitope tags with their binding capacity and the slurry volume that captures 10 µg.

Change log

2025-04-13	Benjamin C. Buchmuller	Adaptation as SOP.
2026-05-22	Benjamin C. Buchmuller	Fix transcription error in Ammonium bicarbonate amount (was 1.58 g, now 1.98 g — recipe v1 delivered 0.4 M instead of the declared 0.5 M; corrected recipe yields the intended 0.5 M stock in 50 mL).
2026-09-07	Benjamin C. Buchmuller	Scope narrowed to soluble targets; chromatin-bound histones and nucleosomes moved to the native chromatin protocol. Corrected the EDTA ingredient of the Nuclear IP buffer, which pointed at the EGTA stock.

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