Immunoprecipitation of soluble proteins

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

This is a bench card. Full protocol available online.

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	SOP 0002-A	150 mM	None		General IP buffer
Cytosol and Membranes	Mild detergent lysis	SOP 0002-B	150 mM	None		General IP buffer
Cytosol	Hypotonic lysis, nuclei removed	SOP 0001-A	150 mM	None		General IP buffer
Nucleosol	Low-salt nuclear extraction	SOP 0025	0–75 mM	3+1 to 6+1 high-salt buffer		General IP buffer
Nucleus	Total chromatin extraction	SOP 0025-B	300–600 mM	1+1 to 1+3 low-salt buffer		Nuclear extract IP buffer
Chromatin-associated proteins	Partial MNase digestion, salt fractionation	SOP 0025-B	300–600 mM	1+1 to 1+3 low-salt buffer		Nuclear extract IP buffer
Histones, acid-extracted	Acid extraction	SOP 0026	No salt	Dialyze	No	Nuclear extract IP buffer
Nucleosomes	Full MNase digestion, chelator release	SOP 0025-A	No salt	Not applicable	No	Not applicable

(2.) Decide whether to crosslink before harvest. Crosslinking is optional for soluble targets, but must not be used before acid extraction of histones SOP 0026.

(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.

> ***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:

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- 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.

⊞ Optional: *Crosslinking with formaldehyde*

(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.

(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

(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*

(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.

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› *Preparation of antibody-coated beads*

(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.

(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.

(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.

› *Preparation of affinity resin for tagged baits*

(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.

> ***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. 1 h

(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.

›› *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 🕮

(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. 🐞 🕮

A › ***Elution in sample buffer***

(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 🕮

B › ***Elution under acidic conditions***

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

> ***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.) 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 🕮

(4.) Pellet the beads as before. Transfer the clear supernatant to a new tube.

(5.) Repeat the elution at least once to increase recovery.

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