Step 1: Bind Biotinylated Proteins to Beads

Use Dynabeads™ MyOne Streptavidin C1 (or T1).

Lysis Buffer:

50 mM Tris-HCl pH 7.5
150 mM NaCl
0.1–0.5% NP-40 or Triton X-100
1 mM EDTA
Protease inhibitors (EDTA-free if downstream metal steps)

Notes:

- Avoid: SDS >0.1% (kills binding, MS cleanup problems), Biotin, avidin, serum and excess glycerol (>5%)
- nuclear / chromatin: add Benzonase (25–50 U/mL) which reduces DNA-mediated background

Biotinylated Protein binding to beads:

- Pre-wash beads 3× in lysis buffer
- Bead:Protein Ratio: 10–30 µL bead slurry per mg lysate protein
- Incubate 30–60 min at RT or 2 h at 4 °C - gentle rotation only

Step 2: Protein Removal from DynaBeads

Use on-bead digestion for MS – this provides the best peptide recovery with the lowest streptavidin contamination.

1. Wash beads (critical for MS cleanliness)

Use stringent washes to remove nonspecific binders.

a. Lysis buffer ×2

b. followed by high salt wash:

- 50 mM Tris pH 7.5
- 500 mM NaCl
- 0.1% NP-40 or Triton X-100

c. Detergent wash:

- 50 mM Tris pH 7.5
- 150 mM NaCl
- 0.5% NP-40

d. Chaotrope wash (can be optional)

- 2 M urea
- 50 mM Tris pH 7.5

e. Final MS-compatible buffer wash in 50 mM ammonium bicarbonate (ABC)

2. Reduction

a. Add 5–10 mM DTT
b. Incubate beads 30 min at 56 °C
c. Briefly spin and magnetize beads

3. Alkylation

a. Add 10–20 mM iodoacetamide
b. Incubate 30 min at room temperature, dark
c. Quench with an extra 5 mM DTT

4. Protease digestion (on beads)

a. Add Trypsin or Lys-C Enzyme:protein ~1:50–1:100
b. Incubate overnight at 37 °C with gentle shaking

c. Optional (recommended): - Add a second small aliquot of trypsin
Digest an additional 2–4 h

5. Collect peptides
a. Magnetize beads and transfer supernatant.
b. (Optional: rinse beads once with 50 mM ABC and combine)

6. Peptide cleanup
a. Acidify with 0.5–1% formic acid
b. Desalt using C18 StageTips or cartridges
c. Dry and resuspend for LC-MS/MS

Notes specific to biotin photo-crosslinkers TFPA-PEG-biotin and benzophenone-biotin

- Crosslinks survive reduction/alkylation
- Modified peptides may stay bead-bound → that's fine
- Goal is protein ID, not mapping the adduct