Oct 22, 2025

Co-Immunoprecipitation/Pull-Down of C1q-Interacting Intracellular Proteins/Protein Complexes for Unbiased Forward Screen Using NanoLC-MS/MS

Nature Communications

DOI
https://dx.doi.org/10.17504/protocols.io.14egnrydpl5d/v1

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Protocol Citation: Katja Piltti 2025. Co-Immunoprecipitation/Pull-Down of C1q-Interacting Intracellular Proteins/Protein Complexes for Unbiased Forward Screen Using NanoLC-MS/MS. **protocols.io** https://dx.doi.org/10.17504/protocols.io.14egnrydpl5d/v1

Manuscript citation:
Piltti KM et al. C1q drives neural stem cell quiescence by regulating cell cycle and metabolism through BAI1. Nature Communications (In press)

Protocol status: Working
We use this protocol and it's working

Created: October 21, 2025

Last Modified: October 22, 2025

Protocol Integer ID: 230351

Keywords: Co-immunoprecipitation (Co-IP), Pull-down assay, Discovery proteomics, Protein–protein interaction mapping, NHS-SS-Biotin biotinylation, DSP crosslinker, Streptavidin magnetic beads, Peptide identification, Protein complex capture, Intracellular binding partners, Neural stem cells, Stem cell biology, Proteomic workflows, LC-MS/MS preparation, protein complexes for unbiased forward screen, interacting intracellular protein, protein complex, using nanolc, crosslinker for an unbiased forward screen, permeable biotin, using cell membrane, biotin, unbiased forward screen, cell membrane, down of c1q, protein, c1q

Funders Acknowledgements:
Wings for Life funding
Grant ID: WFL-US-01/18

Abstract

Co-immunoprecipitation/pull-down of C1q-interacting intracellular
proteins/protein complexes using cell membrane-permeable biotin and crosslinker for an unbiased forward screen using nanoLC-MS/MS.

Materials

- Human C1q (MyBioSource, MBS147305)
- Zeba spin desalting column (7K MWCO, ThermoFisher Scientific)
- NHS-SS-Biotin (ThermoScientific, PI21441)
- PBS
- BCA analysis kit
- Poly-L-ornithine and laminin coated 6-well plates
- DSP (ThermoScientific, PI22586)
- Pierce IP lysis buffer (ThermoScientific, PI87787)
- Pierce Streptavidin Magnetic beads (ThermoScientific, PI188816)
- Protease and phosphatase inhibitors

Prepare Biotin-Labeled C1q

1 Resuspend 1 mg of purified human C1q (MyBioSource, MBS147305) in 1 mL Milli-Q H2O (resulting in 1 mg/mL concentration).

2 Buffer exchange 500 µg of C1q to PBS using a Zeba spin desalting column (7K MWCO, ThermoFisher Scientific), following the manufacturer's instructions.

Biotinylation Reaction

3 Incubate C1q in PBS (1.25 nM) with a 20-fold molar excess (25 nM) of NHS-SS-Biotin using the Ez-link NHS-SS-Biotinylation kit (ThermoScientific, PI21441) for 1 hour at room temperature with agitation.

Remove Non-bound Biotin

4 Perform a buffer exchange using a Zeba spin desalting column (7K MWCO, ThermoFisher Scientific) to achieve a final volume of 500 µL.

Concentration Determination

5 Use a 10 µL aliquot (1:30 dilution) for BCA analysis to determine C1q protein concentration.

6 Expect a recovery of 60-80% of the original C1q amount after biotinylation.

Verification

7 Analyze an aliquot of biotinylated C1q by Western blot to confirm successful biotinylation.

Cell Treatment and Cross-linking

8 Grow hNSC as a monolayer on poly-L-ornithine and laminin coated 6-well plates until 80% confluence.

9 Wash the cells three times with PBS (pH 7.2).

C1q Treatment

10 Add 200 nM of biotinylated C1q (bait) or an equal volume of growth medium (non-treated control) to the cells.

11 Incubate for 15 or 30 minutes at room temperature.

Intracellular cross-linking

12 Wash cells three times with PBS (pH 7.2) and add 2 mM cell-permeable DSP on the cells (ThermoScientific, PI22586) for 30 minutes at room temperature.

13 Quench the reaction with three 5-minute washes of 25 mM Tris in PBS.

Cell Lysis and Protein Pull-down

14 Lyse cells using Pierce IP lysis buffer (ThermoScientific, PI87787), supplemented with protease and phosphatase inhibitors, on ice.

15 Centrifuge lysates at 13,000g for 10 minutes at 4°C.

Protein Pull-down

16 Incubate supernatants with washed Pierce Streptavidin Magnetic beads (ThermoScientific, PI188816) for 1 hour under slow, low-speed rotation at room temperature.

17 Wash to remove non-specific binding partners.

18 See the “Denaturing On-bead Digestion for LC-MS/MS”-protocol compatible with this crosslinking method for next steps.