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Immunoprecipitation for Flag-tagged proteins

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Protocol status: Working

We use this protocol and it's working

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Abstract

Immunoprecipitation for Flag-tagged proteins for various biomolecular analyses.



Transfection and collection

- 1 Transfect HEK293T cells with 2µg of plasmid expressing Flag-tagged protein of interest using enhanced polyethyleneimine transfection reagent in Opti-MEM (Thermo Fisher Scientific, 31985070).
- 2 After 48h, collect cells and lyse with IP Lysis Buffer (25 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% NP40, 5% Glycerol).

Prepare beads

- 3 Defrost beads on ice. Wash 3x with IP Lysis Buffer.

IP

- 4 Use 3% of the collected sample for the 'Input'.
- 5 Incubate the remaining sample with Anti-FLAG M2 Magnetic beads (Millipore Sigma, M8823) for 1 h at 4 °C in a revolving rack.
- 6 Place tubes in magnetic rack and collect 3% the supernatant as the "Flow-through" sample. Aspirate the remaining flow-through.
- 7 Wash beads with IP lysis buffer.
- 8 Add 2x Laemmli buffer (Bio-Rad, 1610747, Hercules, CA, USA) with 20% 2-mercaptoethanol (Bio-Rad, 1610710) to the beads.
- 9 Vortex, then incubate for 15 min at 75 °C to elude proteins from the beads.
- 10 Place tubes in magnetic rack and collect elution.



- 11 Run all samples on SDS-PAGE gel and visualize with method of choice.