FEB 28, 2024

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.q26g7poxqgwz/v1

Protocol Citation: Jean-Louis Parmasad 2024. Immunoprecipitation for Flag-tagged proteins. **protocols.io** https://dx.doi.org/10.17504/protocols.io.q26g7poxqgwz/v1

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

Created: Feb 28, 2024

Immunoprecipitation for Flag-tagged proteins

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ABSTRACT

Immunoprecipitation for Flag-tagged proteins for various biomolecular analyses.

Last Modified: Feb 28, 2024

PROTOCOL integer ID: 95879

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% NP4O, 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.