

Anti-Flag Co-Immunoprecipitation

(From JLA lab protocol)

1. Wash cells carefully with 10 or 25 ml PBS (10 or 15 cm plates, respectively).
2. Add 5 or 12 ml PBS and scrape off cells using a rubber policeman. Spin 1,000 rpm for 10 min.
3. Resuspend in ice-cold 1 ml hypotonic lysis buffer and transfer to eppendorf tubes, and incubate on ice, 10 min.
4. Add NaCl to 150 mM (optional: + RNaseA to 125 µg/ml). Incubate on ice 5 min.
5. Spin 14,000 rpm, 4°C, 15 min.
6. Transfer 50 µl supernatant to to 50 µl SDS LB (Total extract control). Store at -20°C.
7. Transfer remainder of supernatant to tube containing α-Flag-M2 agarose slurry (10 cm plates: use 40 µl α-Flag-M2 agarose; 15 cm plates: use 80 µl) which has already been washed twice with 1 ml NET-2 (1,000 rpm, 1 min for each wash). Optional: add low concentration of Flag peptide (0.5 – 2 µg/ml) to out compete any unspecific binding.
8. Wrap tubes with parafilm and nutate at 4°C, ≥ 2h.
9. Wash beads 8 times with 1 ml NET-2 (ice-cold). Spin 1,000 rpm, 1 min for each wash.
10. After last wash, remove all wash buffer with pipette.
Either:
 - a) Add SDS LB to beads/pellet (10 cm plates: 20 µl SDS LB; 15 cm plates: 40 µl), or
 - b) Elute by incubating at 4°C for ≥ 2h with shaking in NET-2 (20µl for 10 cm plates; 40 µl for 15 cm plates) with 0.2-1 mg/ml Flag peptide. Spin 1,000 rpm, 1 min and transfer supernatant (10 cm plates: 20 µl; 15 cm plates: 40 µl) to a new tube, and spin again (this is to avoid any residual IgG in the final sample). Transfer supernatant to one volume of SDS LB. Load sample on SDS-PAGE or store at -20 or -80°C.

Solutions:

Hypotonic gentle lysis buffer:	10 mM Tris-HCl, pH 7.5 10 mM NaCl 2 mM EDTA 0.5% Triton X-100 1 mM PMSF (prepare and add fresh) 1 µg/ml aprotinin 1 µg/ml leupeptin
NET-2:	50 mM Tris-HCl, pH 7.5 150 mM NaCl 0.05% Triton X-100