ImmunoPrecipitation using GFP-TRAP

Boisvert Lab, December 2020

- Resuspend cell pellet in cold lysis buffer (1 ml lysis buffer/100 mm petri dish).
- Allow lysis to continue 10 minutes on ice.
- Spin down insoluble material 10 minutes at 13,000 rpm, or until no pellet is detected after centrifugation.
- Add GFP-TRAP sepharose to lysate (10-20 µl of washed beads per 100 mm petri dish).
- Tumble 2 hrs at 4^{o}C.
- Spin down resin at 2,000 rpm for 3 minutes.
- Wash three times with lysis buffer.
- Wash twice with PBS 1X.
- Spin down resin and aspirate all liquid.
- Add Laemmli loading buffer to elute.
- Boil sample for 5 minutes.
- Load on SDS-PAGE.

Solutions:

Lysis Buffer	For 50 ml
1% Triton X-100	5 ml of 10%
150 mM NaCl	1.5 ml of 5 M
20 mM Tris-HCl, pH 7.5	1 ml of 1 M
0.1 mg/ml PMSF	500 µl of 10 µg/µl