IMMUNOPRECIPITATION

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PROTOCOL

This protocol is designed for 6 well plates. Scale up or down accordingly for other plate types.

- Pre-work:
  - Take protease inhibitor out of the freezer (Hopper upper)
  - Put NP40 Lysis Buffer on ice
  - Set heat block to 100°C; set microcentrifuge to 4°C
  - Mix 50µl protease inhibitor
    10ml NP40 lysis buffer
    5 mM N-ethylmaleimide (0.025g in 40ml) (bottom of Darwin, prevents deubiquitination)

- Transport plates from the TC incubator to the lab bench
- Aspirate media off of cells; wash with 1x PBS, aspirate off solution
- Add 250µl of NP40 Lysis buffer solution to each well
- Place plates in the -80°C freezer for 10 minutes or until frozen; once frozen, transfer to lab top and wait until thawed
- Scrape plates to remove any cells still adhered to the surface of the plates; transfer to ependorfs
- Spin at 4°C, 13000rpm for 10 minutes (or until a pellet appears)
- Discard pellet by transferring to a new eppendorf tube.
- Add the primary antibody (at whatever concentration is recommended by the manufacturer; if our home grown myc 9E10 use 100µl) to each eppendorf; nutate at 4°C for 1 hour.
- Add 125µl of 10% protein A sepharose beads to each eppendorf; nutate at 4°C overnight.
- Very, very carefully wash 3x with the NP40 lysis buffer solution using a 11-gage needle to rid of the supernatant after each spin.
- Add 30µl of sample buffer (with DTT added if your goal is to reduce your samples); boil for 10 minutes.
- Allow to cool; freeze samples at -20°C or run gel immediately.