

Immunoprecipitation

From a monolayer of cells (100 cm² plate)

- 1.) Remove media. Wash with PBS. Remove all PBS.
- 2.) Add 1 mL ice-cold EBC buffer with protease inhibitors (Complete w/o EDTA) freshly added with phosphatase inhibitors (NaF and NaVO₄). Scrape plate with a cell scraper. Incubate 30 min. on ice.
- 3.) Centrifuge 14,000 RPM for 5 min at 4 C. Transfer supernatant to new tube.
- 4.) To pre-clear lysate, add 20 uL Protein A or G-Agarose (depends on antibody—look at Harlow and Lane to see which conjugate is best for each antibody). Shake at 4 C for 30 min.
- 5.) Pellet beads by centrifugation at 2,500 rpm for 5 min. at 4 C. Transfer supernatant to new tube.
- 6.) Add antibody (I used 1:1000, but depends on Ab). Shake at 4 C for 1 hour to O/N.
- 7.) Add 20 uL Protein A/G-Agarose. Shake at 4 C for 1 hour.
- 8.) Centrifuge at 2,500 rpm for 5 min at 4 C. Discard supernatant.
- 9.) Wash pellet 4 times with EBC buffer with protease and phosphatase inhibitors.
- 10.) After final wash, aspirate and discard supernatant. Resuspend in 20 uL of 1X electrophoresis sample buffer and DTT
- 11.) Boil for 3 minutes.
- 12.) Spin briefly. Load gel (Don't load beads onto gel).

Taken from Santa Cruz Biotechnology method.