C. Barber 2/2002

Immunoprecipitation

From a monolayer of cells (100 cm^2 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.