Immunodepletion

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This is for studying quantitative relationships of proteins in a complex.

Procedure

1. Couple the antisera to protein G beads:
   a. Dilute 100 μl of serum in 1 ml of IP buffer (recipe) with 10mg/ml of BSA and 0.1% NP40.
   b. Add 50 μl of protein G beads (packed volume), rotate on ? at 4 ^{o}C overnight.
   c. Wash the beads with X amount of the IP buffer 3 times.
2. Deplete:

   Mix 1mg total extract diluted in 1ml IP buffer + 20μl protein G beads + 0.05% NP40, rotate on what overnight at 4^{o}C
3. Pellet the protein G beads with a brief spin at 4^{o}C, 4,000g X 1min, take the supernatant and load 20 μl onto SDS-PAGE for western blot.

Reference: Cheng et al (2005) Genes & Development 19:234-241