

Immunodepletion of Hela extract

1. Affiprep-Protein A beads, 3 times PBS wash.
Resuspend in 5V PBS to make 20% beads. Add NaN₃ for long time storage.
 2. 50ul beads suspension (10ul beads)
Add 500 ulPBS w/ 1mg/ml BSA, mix,
Add Ab: 5-10ug, don't forget sham Ab.
rotate O/N in cold room.
 3. Spin gently, 2000rpmx3min.
wash twice with 1 ml PBS/BSA.
Last time, take off sup. as much as possible.
 4. Add 40 ul Hela extract, rotate 2 hrs in cold room. Speed is critical, ~60rpm in the rotator. The goal is that with the set speed, the beads will mix with the lysate, but the lysate can still stay in the bottom of the tube even when the tube is upside down. It will also work if you Leave the tube in ice, and tap intermittently for 1hr.
 5. spin, collect sup. 14000rpmx1min. may need respin to remove beads. Typically recovered 30-35 ul.
 6. Do immunoblot. Take 3ul immunodepleted lysate to run SDS PAGE. Compare to sham. Freeze big frags. at -80.
- Note. 1. Can scale up.
2. Can do 2nd round.