Immunoprecipitation protocol

Wash buffers

10mM HEPES; adjust to pH 7.6
150mM NaCl
5mM MgCl2
1% Triton X-100
Protease inhibitor cocktail

Store up to 6 months at 4°C. Immediately before use add protease inhibitors

Protein A/G beads from Invitrogen dynamic bead

Immunoprecipitation

The typical way for IP is mix antibody with protein sample, then using Protein A/G beads to pull down the antibody with the target protein This method can give us highest yield. Different method may apply in different requirement.

Step:

1. On ice, add 20ul antibody (different amount of antibody may test for best result) into 1ml protein sample in 1.5ml tube.

2. 4°C shake 1to 12hr.

3. Add 20ul protein A/G bead (Invitrogen, dynamic bead)

4. 4°C shake 2 to 4hr.

5. Using magnetic stand to help remove supernatant solution.

6. Wash beads 5 times with wash buffer.

7. Add 40ul 2X SDS-PAGE sample buffer for elution

8. Boil sample with 99°C 20min. then separate soluble part and beads. Target protein and antibody should stay in the soluble part for further analysis.