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IMMUNOPRECIPITATION(IP)

Reagents

1) PBS
2) Protein A or G agarose beads
3) Lysis Buffer
4) Protease Inhibitor: PMSF, or aprotinin
5) Primary Antibody

Sample Preclearing

1) Wash protein A- or G-Sepharose or protein L-Agarose with 10X volume of RIPA buffer.
2) Vortex and centrifuge for 1 minute in a microfuge.
3) Resuspend the pellet in the original volume that the protein A or protein G matrix was in.
4) Add protein A- or G-Sepharose or protein L-Agarose to the sample and incubate at 4°C for 30 minutes with shaking.
5) Centrifuge for 1 minute in a microfuge to pellet absorbed nonspecific proteins and insoluble material. Discard.

Incubation with Primary Antibody

1) Add primary antibody to precleared sample and incubate for 1 hour at 4°C with gentle agitation.
2) Add protein-A or -G Sepharose beads and incubate 1 hour at 4° C with gentle agitation.
3) Centrifuge for 1 minute in a microfuge. Wash the pellet in 1 ml of RIPA buffer.
4) Repeat twice.

Removal of Antibody-antigen Complex

1) Resuspend immunoprecipitate in a buffered solution with either 1% SDS and 15 mM beta-mercaptoethanol or 8M urea.
2) Heat at 90-100°C for 5-20 minutes with occasional vortexing.
3) Centrifuge for 1-2 minutes in a microfuge.
4) The supernatant is ready to be analyzed by Western blot.