Immunoprecipitation (IP) Protocol

The combined procedures of immunoprecipitation and SDS-PAGE can be a powerful tool to assess the amount and size of an antibody-reactive antigen present in a complex protein mixture. The basic protocol uses a primary antibody followed by a secondary antibody-agarose conjugate to immunoprecipitate the antigen.

I. Reagents Required

Reagent	Preparation
Tris Buffered Saline	Use 10X TBS, pH 7.5 (1.0 M Tris HCl, 1.5 M NaCl) (MB-012). Dilute appropriate volume to 1X with deionized water. Store at room temperature up to one month.
Lysis Buffer	TBS containing 1.0% of an appropriate detergent (i.e. Triton X-100), 1 mg/mL bovine serum albumin (BSA) (BSA-10), and appropriate proteinase inhibitor.
Dilution Buffer	Same as Lysis Buffer except without proteinase inhibitor.
Agarose Conjugates for Lysate Pre-Treatment	Pre-adsorb lysate to remove non-specific binding to primary and secondary antibodies. Use agarose normal IgG from the same species as the primary antibody and the host secondary antibody. Prepare washed slurry at 1:1 using dilution buffer.
Control for Primary Antibody	For polyclonal antiserum, use non-immune serum from the same species. For monoclonal, use the same isotype and purity.
Agarose Conjugates for Immunoprecipitation	Use agarose secondary antibody conjugate against the same species as the primary antibody. Prepare washed slurry at 1:1 using dilution buffer.
Tris Buffer	Prepare 0.05 M Tris buffer, pH 6.8.
2X SDS-PAGE Sample Buffer (MB-018)	N/A
2-Mercaptoethanol	N/A

II. Procedure

1. Prepare lysate by incubating 5 x 10^7 cells in Lysis Buffer for 30-60 minutes on ice.
2. Vortex lysate and centrifuge for 10 minutes at 250 x g to remove nuclei. Retain supernatant.
3. Clarify supernate by centrifugation for 30 minutes at 100,000 x g or microcentrifuge for 30 minutes at 10,000 x g.
4. Pretreat lysate to remove nonspecific protein binding by adding agarose conjugates. Use 10 µL of control agarose per 200 µL lysate. Shake for 1 hour at 4°C. Centrifuge at 200 x g. Save supernatant.
5. Add 200 µL of pretreated lysate containing antigen to each of two microfuge tubes. Bring volume to 1 mL with dilution buffer.
6. Add primary antibody to one tube. For polyclonal antiserum or ascites fluid use 0.5-5.0 µL. For tissue culture supernatant use 10-100 µL. To the second tube add an equivalent volume of control for primary antibody. Incubate on ice for 1 hour.
7. For immunoprecipitation add 50 µL of agarose conjugate per tube. Mix with gentle shaking for 1 hour at 4°C.
8. Centrifuge tube 1 minute at 200 x g or microcentrifuge for 5 seconds. Carefully remove the supernatant with a pipette. Gently resuspend pellet in 1 mL dilution buffer. Repeat wash. Follow with a wash in TBS and then a final wash in 0.5 M Tris, pH 6.8.
9. Centrifuge again as above. Add 20-50 µL of sample buffer. Mix and heat for 5 minutes at 100°C. Microcentrifuge briefly and apply supernatant directly to nonreducing SDS-PAGE. If reducing conditions are desired, transfer the supernatant to a new tube and add 5% 2-mercaptoethanol. Mix and heat as above.
10. Electrophorese protein mixture. Stain gel or immunoblot to visualize. Bands present will include polypeptides of antigen and antibodies used.