ROCKLAND™
antibodies & assays

Immunoprecipitation

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.

Reagents Required

- Tris Buffered Saline. Use 10X TBS, pH 7.5 (1.0 M Tris HCl, 1.5 M NaCl) Code # 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, code BSA-10), and an appropriate proteinase inhibitor.
- Dilution buffer. Same as lysis buffer 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.

Primary antibody.

- Control for primary antibody. For polyclonal antiserum, use nonimmune serum from the same species. For monoclonal antibodies, 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. Use SDS-PAGE Sample Buffer Code # MB-018.
- 2-Mercaptoethanol.

Procedure

1. Prepare lysate by incubating 5 x 107 cells in lysis buffer for 30 to 60 min on ice.
2. Vortex lysate and centrifuge for 10 min at 250 x g to remove nuclei. Retain supernate.
3. Clarify supernate by centrifugation for 30 min at 100,000 x g or microcentrifuge for 30 min at 10,000 x g.
4. Pretreat lysate to remove nonspecific protein binding by adding agarose conjugates. Use 10 ul of control agarose per 200 ul lysate. Shake for 1 h at 4° C. Centrifuge at 200 x g. Save supernatant.
5. Add 200 ul 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 to 5.0 ul. For tissue culture supernatant use 10 to 100 ul. To the second tube add an equivalent volume of control for primary antibody. Incubate on ice for 1 h.
7. For immunoprecipitation add 50 ul of agarose conjugate per tube. Mix with gentle shaking for 1 h at 4° C.
8. Centrifuge tube 1 min at 200 x g or microcentrifuge for 5 seconds. Carefully remove the supernate 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 to 50 ul of sample buffer. Mix and heat for 5 min at 100° C. Microcentrifuge briefly and apply supernate directly to nonreducing SDS-PAGE. If reducing conditions are desired, transfer the supernate 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.