

## Protocol

# Immunoprecipitation (IP)

The combined procedures of Immunoprecipitation (IP) 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 IP the antigen.

## Reagents Required

Product	Preparation	Item No.
Tris Buffered Saline (TBS)	Use 10X TBS, pH 7.5 (1.0 M Tris HCl, 1.5 M NaCl). Dilute appropriate volume to 1X with deionized water. Store at room temperature for up to one month.	MB-012
Lysis Buffer	TBS containing 1.0% of an appropriate detergent (i.e. Triton X-100), 1 mg/mL Bovine Serum Albumin (BSA), 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 a matching isotype control with the same purity.	
Agarose Conjugates (for IP)	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		KBB-001
β-mercaptoethanol		

## Procedure

1. Prepare lysate by incubating  $5 \times 10^7$  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 supernate.
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  $\mu$ L of control agarose per 200  $\mu$ L lysate. Shake for 1 hour at 4°C. Centrifuge at 200 x g. Save supernatant.
5. Add 200  $\mu$ L 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  $\mu$ L. For tissue culture supernatant use 10 to 100  $\mu$ L. To the second tube, add an equivalent volume of control for primary antibody. Incubate on ice for 1 hour.
7. For IP, add 50  $\mu$ L 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  $\mu$ L of sample buffer. Mix and heat for 5 minutes 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%  $\beta$ -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.

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