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## IMMUNOPRECIPITATION (IP) - PROTOCOL

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### Principle

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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.

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### Reagents Required

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#### Cell Preparation:

- Tris Buffered Saline. Use a 10x TBS, pH 7.5 (1.0M Tris HCl, 1.5M NaCl). Dilute appropriate volume to 1x with de-ionized water. Store at room temperature up to one month.
- Lysis Buffer. TBS containing 1.0% of an appropriate detergent, 1mg/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-absorb lysates 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 0.05M Tris buffer, pH 6.8.
  - 2x SDS-PAGE Sample Buffer
  - 2-Mercaptoethanol
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### Procedure

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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 micro-centrifuge for 30 minutes at 10,000 x g.
4. Pre-treat lysate to remove nonspecific protein binding by adding agarose conjugate. 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 0.5-5  $\mu$ l. For tissue culture supernatant, use 10-100 $\mu$ l. To the second tube, add an equivalent volume of control for primary antibody. Incubate on ice for one hour.
7. For immuno-precipitation add 50 $\mu$ l of agarose conjugate per tube. Mix with gentle shaking for 1 hour at 4 $^{\circ}$ C.
8. Centrifuge tube 1 minute at 200 x g or micro-centrifuge for 5 seconds. Carefully remove the supernate with a pipette. Gently resuspend pellet in 1ml dilution buffer. Repeat wash. Follow with a wash in TBS and then a final wash in 0.5M 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  $^{\circ}$ C. Micro-centrifuge briefly and apply supernate directly to non-reducing 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.