Protein Immunoprecipitation

Binding Antibody to Protein-G Sepharose

<table>
<thead>
<tr>
<th>Antibody Type</th>
<th>Volume</th>
<th>Sepharose (volume of 50% slurry)</th>
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</thead>
<tbody>
<tr>
<td>mAb</td>
<td>2 - 4 µl ascites</td>
<td>30 µl Protein G</td>
</tr>
<tr>
<td>pAb</td>
<td>1 - 2 µl serum</td>
<td>30 µl Protein A</td>
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1. Wash sepharose beads ("beads") 3 X with IP buffer + 0.1% NP-40 ("IP/NP40").
   - IP buffer: 50 mM Tris-HCl, pH 8, 150 mM NaCl.
   - Collect beads by microcentrifugation for 30 seconds at 2000 rpm. Carefully remove as much supernatant as possible. Leave some supernatant behind to prevent aspiration of beads.

2. Resuspend beads in 1 ml of IP/NP40. Add monoclonal antibody. Rotate for 1 hour at 4°C.

3. Wash beads with IP/NP40: 2 quick washes, then 3 X 5 minutes (on rotator). Store beads on ice until needed.

Immunoprecipitation From Yeast Cells

1. Plan for ~5 OD units of cells per IP. Chill cells on ice. Wash cells with cold IP/NP40 + protease inhibitor cocktails ("IP/NP40/PICs"). Washed cells may be frozen until use. If starting with frozen pellet, thaw quickly by addition of buffer in step 2 and immediately go to step 3.

2. Resuspend up to 25 OD units in 0.5 ml IP/NP40/PICs + 0.1 mM DTT ("IP/NP40/PICs/DTT").

3. Add 0.25 g glass beads (0.5 mm) in 2 ml microfuge tube and place on vortex mixer at 4°C for 30 minutes. This will yield ~75% lysis of cells.

4. Transfer lysate to new tube. Wash glass beads with 0.5 ml IP/NP40/PICs/DTT. Pool.

5. Microfuge 5 minutes at top speed at 4°C (1 ml aliquots). Transfer supernatant to fresh tube. Leave some supernatant behind to avoid transfer of pellet.
   - Optional preclearing step. Transfer supernatant to washed beads (no Ab). Rotate 1 hour at 4°C.

6. Repeat spin in step 5. Save supernatant (25 µl) for "total" sample lane.

7. Immunoprecipitation: Transfer supernatant to beads. Leave some supernatant behind to avoid transfer of cell debris pellet. Rotate for 1 hour at 4°C.

8. Spin down beads. Wash with ice cold IP/NP40/PICs/DTT. Do 2 quick washes, then 3 X 5 minute washes. Rotate at 4°C during 5 minute washes. Then do 2 quick washes with cold IP buffer alone (to remove NP40). Beads with minimal residual buffer may be frozen and stored at -80°C.
   - Add equal volume of 2X SDS-PAGE sample buffer, heat in boiling water bath 2 minutes, spin in microfuge, load gel.