General Protocol Immunoprecipitation

Typically, complete immunoprecipitation of radio-labeled antigen from extracts of transfected mammalian cells requires between 1-5 µL of polyclonal antiserum, 5-100 µL of hybridoma tissue culture medium, or 1-3 µL of ascites. If more antibody is used than is necessary, non-specific background will increase. The concentration of immunoglobulins in antiserum is approximately 6-8 mg/mL. Supernatant from cultured hybridoma cells typically contains 10-100 µg/mL immunoglobulin, and ascites fluid contains 5-7 mg/mL immunoglobulin.

Step 1 Divide the preparation of antigen into two equally sized aliquots and place in microfuge tubes. Adjust the volume of each aliquot to 0.5 mL with I.P. Buffer*. To one aliquot, add antibody directed against the target protein. To the other aliquot, add the same volume of a control antibody. Gently rock both aliquots for 1 hour at 4°C.

Step 2 Add Protein-G Sepharose to the antigen-antibody mixture and incubate for 1 hour at 4°C on a rocking platform.

Step 3 Centrifuge the Protein-G Sepharose antibody-antigen complex at 10,000 × G for 20 seconds at 4°C in a microfuge. Remove the supernatant by gentle aspiration. Add 1 ml of Wash Buffer** and re-suspend the Sepharose beads.

Step 4 Incubate the re-suspended beads for 20 minutes at 4°C on a rocking platform. This allows time for the I.P. buffer to equilibrate with the fluid trapped between the beads.

Step 5 Repeat steps 3 and 4 three times. Collect the final washed Protein-G Sepharose antibody-antigen complex by centrifugation at 10,000 × G for 20 seconds at 4°C in a microfuge. Take care to remove the last traces of the final wash.

Step 6 Add reducing gel-loading buffer and boil for 3 minutes.

Step 7 Remove the Protein-G Sepharose from the complex by centrifugation at 10,000 × G for 20 seconds at room temperature in a microfuge. Transfer the supernatant to a fresh tube and separate the sample by method of electrophoresis.

Nordic Biosite AB
Propellervägen 4A | 183 62 TÄBY, SWEDEN
+46 (0)8 5444 3340 | info@nordicbiosite.com

*IP Buffer

50 mm Tris HCl (pH 7.5)

150 mM NaCl

0.1% Tween 20 (or 0.1% Nonidet P 40 (NP 40))

1 mM EDTA (pH 8.0)

0.25% Gelatin

**Wash Buffer

50 mm Tris HCl (pH 7.5)

150 mM NaCl

0.1% Tween 20 (or 0.1% Nonidet P 40 (NP 40))

1 mM EDTA (pH 8.0)

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