Immunopurification — Small Scale using Epitope Tag Affinity Matrices

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Abstract

Immunochemistry Protocols: Immunopurification - (Small Scale) Using Anti-Epitope Tag Affinity Matrices:

Typically, immunoprecipitation of 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, nonspecific 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.

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Guidelines

***IP Buffer**
• 50 mm Tris HCl (pH 7.5)
• 150 mM NaCl
• 0.1% Tween 20 (or 0.1% Nonidet P 40)
• 0.5% BSA
• 1 mM EDTA (pH 8.0)
• 1mM β-mercaptoethanol (see Considerations)
• 1mM phenlymethylsulfonyl fluoride (PMSF) or protease inhibitors of choice

****Wash Buffer**
• 50 mm Tris HCl (pH 7.5)
• 150 mM NaCl
• 0.1% Tween 20 (or 0.1% Nonidet P 40)
• 1 mM EDTA (pH 8.0)
• 1mM phenlymethylsulfonyl fluoride (PMSF) or protease inhibitors of choice

Considerations:
- The ingredient β-mercaptoethanol is not always necessary but is included in the immunopurification buffer to improve the solubility of the proteins in the lysate. At the concentration indicated, it should not reduce antibodies. DTT may be used as a substitute.
- The suggested immunopurification buffer is by no means the only one that will work. A wide variety of other common physiologic buffers may be employed instead.
- At step 4, if elution is not desired, bound protein may be visualized on a gel by resuspending beads directly in reducing gel loading buffer, boiling for 5 minutes, and loading the supernatants on a gel for analysis. Note that the loading buffer may release a portion of the antibodies from the affinity matrix.

Protocol

Step 1.
Combine affinity matrix and IP Buffer in a microfuge tube at a 1:5 dilution. Add tagged protein to mixture.Gently rock aliquots for one hour at 4°C.

Step 2.
Centrifuge mixture at 10,000g for 20 seconds at 4°C. Remove supernatant without disturbing beads. Add anequal volume of IP Wash Buffer to the beads and re-suspend matrix. Gently rock aliquots for 20 min. at 4°C.Keep a portion of the supernatant from each rinse step to use in Western blot analysis.

Step 3.
Repeat step 2 four times.

Step 4.

Elute the bound protein with the appropriate epitope peptide at 1 mg/ml concentration in 50 mm Tris HCl(pH 7.5), 50 mM NaCl, 1 mM EDTA (pH 8.0).

Resuspend beads, incubate, centrifuge and withdrawsupernatant as in step 2, repeating for a total of four elutions.

Recover as much of the eluate as possibleat each stage.

Step 5.
For each elution sample, prepare at 1:1 dilution with reducing gel loading buffer. Boil tubes for 3 min.

Step 6.
Analyze the supernatant samples by Western blot.