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NBP1-47601 Protocol

Immunoprecipitation Protocol (NBP1-47601)

Immunoprecipitation
The work can be performed in 1.5 ml micro-centrifuge tubes or in spin columns.

1. Thoroughly resuspend the Anti-Myc Agarose by inverting the tube or vial several times.
2. Add 20-50 l 50% slurry of Anti-Myc Agarose into cell lysate using a widebore pipette tip.
The lysate should be fresh, and for a well expressed tagged protein, 200 l lysate (200-500 g total protein) usually yields a good IP result.
3. Incubate with gentle mixing for 2 h to overnight at 4C.
4. Wash the beads with 1 ml TBS buffer or lysis buffer, such as RIPA (50 mM Tris HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% NP-40, 0.5% sodium deoxycholate), centrifuge for 3 min at 2,000× g, and discard the supernatant. Wash 3 times, avoid losing beads during washes.
5. Elution of the Myc tagged protein.

Option 1. Elution with elution buffer.
Add 30-50 l elution buffer to the beads, gently tap the tube to mix well, centrifuge for 3 min, transfer the supernatant very carefully to a fresh tube (Avoid transferring any beads).
*Note: Neutralize the eluant immediately by add 1 l of 1.5 M Tris, pH 9.0 per 20 l Elution buffer.

Option 2. Elution with Myc peptide
Add 30-50 l Myc peptide solution (100 g/ml Myc peptide in TBS buffer), gently tap the tube to mix well, incubate for 10 min, centrifuge for 3 min, and transfer the supernant to a fresh tube. TBS buffer: 50 mM Tris HCl, 150 mM NaCl, pH 7.4.

Option 3. Elution with SDS loading buffer
Add 30 l 2 SDS loading buffer, gently tap the tube to mix well, boil at 100 for 5 min, centrifuge for 3 min, transfer the supernatant to a fresh tube.
*Note: in this case, the supernatant contains not only the binding proteins, but also IgG (heavy and light chains).

6. Prepare SDS-PAGE gel for western blotting or proceed to other assays.