Spin column protocol for Immunoprecipitation and Glycine elution of proteins from Nano-Trap® agarose beads

Only for research applications, not for diagnostic or therapeutic use.

Harvest cells

For one immunoprecipitation reaction the use of ~10^6 - 10^7 mammalian cells (approx. one 10-cm dish) expressing a protein of interest is recommended. To harvest adherent cells, aspirate growth medium, add 1 ml ice-cold PBS to cells and scrape cells from dish. Transfer cells to a pre-cooled tube, spin at 500 g for 3 min at +4°C and discard supernatant. Wash cell pellet twice with ice-cold PBS, gently resuspending the cells. After washing:

Lyse cells

1. Resuspend cell pellet in 200 µl ice-cold lysis buffer by pipetting or using a syringe.

   note: Supplement lysis buffer with protease inhibitors and 1 mM PMSF (not included).

   optional for nuclear/chromatin proteins: Use RIPA buffer supplemented with 1 mg/ml DNase, 2.5 mM $MgCl_2$, protease inhibitors and 1 mM PMSF (not included).
2. Place the tube on ice for 30 min with extensively pipetting every 10 min.
3. Centrifuge cell lysate at 20.000x g for 10 min at +4°C. Transfer supernatant to a pre-cooled tube. Add 300 µl dilution buffer to lysate. Discard pellet.

   note: At this point cell lysate may be put at -80°C for long-term storage.

   optional: Add 1 mM PMSF and protease inhibitors (not included) to dilution buffer.

Equilibrate beads

4. Remove the upper cap of a new spin column and snap of the plug from the bottom of the spin column (Keep cap and plug!). Place the spin column in a 2 ml tube. Vortex Nano-Trap®_A beads and pipette 25 µl bead slurry into the spin column. Immediately add 500 µl ice-cold dilution buffer. Centrifuge at 100x g for 5-10 sec. Discard flow-through and repeat wash twice. Close column with the bottom plug.

Bind proteins

5. Add diluted lysate (step 3) to equilibrated Nano-Trap®_A beads (step 4). If required, save 50 µl of diluted lysate for immunoblot analysis. Secure the top of the spin column. Tumble end-over-end for 1 hour at 4°C.
6. Remove the bottom cap from the spin column and place it in a new 2 ml tube. Centrifuge at 100x g for 5-10 sec. If required, save 50 µl flow-through for immunoblot analysis. Discard remaining flow-through.

Wash beads

7. Resuspend Nano-Trap®_A beads in 500 µl ice-cold dilution buffer. Place spin column in a 2 ml tube and centrifuge at 100x g for 5-10 sec. Discard flow-through and repeat wash twice. Close column with the bottom plug.

   optional: Increase salt concentration in the second washing step up to 500 mM.

Elute proteins

8. Add 50 µl elution buffer (0.2 M glycine, pH 2.5) to Nano-Trap®_A beads. Pipette Nano-Trap®_A beads up and down for 30 sec. Remove bottom plug of the spin column and place it in a new 2 ml tube containing 5 µl 1M Tris base pH 10.4. Centrifuge at 1000x g for 30-60 sec. To increase elution efficiency this step can be repeated.

Support Please refer to our FAQ section at *www.chromotek.com* or contact *support@chromotek.com*