Spot-Trap Agarose for immunoprecipitation of Spot-Tag fusion proteins from mammalian cell extract

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

Introduction

Epitope tags are useful for the labelling and detection of proteins using immunoblotting, immunoprecipitation, and immunostaining techniques. Because of their small size, they are unlikely to affect the tagged protein's biochemical properties. The novel "Spot-Tag" system consists of a short 12 amino acid affinity tag and a 14.7 kDa small, monovalent, high affinity monoclonal alpaca antibody ("nanobody"). It has been tested for multiple applications and works efficiently in various systems, such as bacteria, yeast, mammalian cell lines, and insect cells. For biochemical analysis including mass spectrometry and enzyme activity measurements, Spot-Tag-fusion proteins and their interacting factors can be isolated fast and efficiently by immunoprecipitation using the Spot-Trap. Spot-Trap utilizes small recombinant alpaca single domain antibody fragments covalently coupled to the surface of agarose beads.

Specificity

The anti-Spot-Tag alpaca monoclonal antibody is highly specific to the Spot-Tag sequence PDRVRAVSHWSS.

Content

Reagent	Code	Quantity
Spot-Trap Agarose	eta-20	20 reactions (0.5 ml resin)

Bead properties

Bead size: ~ 90 µm (cross-linked 4% agarose beads)
Storage buffer: 20% EtOH

Stability and Storage

Shipped at ambient temperature. Upon receipt store at +4°C.
Stable for 1 year. Do not freeze.

Required solutions

Suggested buffer composition

Buffer	Composition
Lysis buffer (CoIP)	10 mM Tris/Cl pH 7.5; 150 mM NaCl; 0.5 mM EDTA; 0.5% NP-40, 0.09% Na-Azide
RIPA buffer	10 mM Tris/Cl pH 7.5; 150 mM NaCl; 0,5 mM EDTA; 0.1% SDS; 1% Triton X-100; 1% Deoxycholate, 0.09% Na-Azide
Dilution buffer	10 mM Tris/Cl pH 7.5; 150 mM NaCl; 0.5 mM EDTA
Spot Wash buffer	10 mM Tris/Cl pH 7.5; 150 mM NaCl; 0.5 mM EDTA, 0.2% Triton X-100, 0.2% Deoxycholate, 0.018% Na-Azide
Spot-Tag peptide	reconstitute Spot-Tag peptide (ep-1) in PBS to a final concentration of 2 mg/ ml (1.4 mM)
2 x SDS-sample buffer	120 mM Tris/Cl pH 6.8; 20% glycerol; 4% SDS, 0.04% bromophenol blue; 10% β-mercaptoethanol

Note: For other cell types like yeast, plants, drosophila, etc. please use your equivalent cell lysis buffer.

Related products

Spot-Tag Toolbox	Code
Spot-Trap Agarose	eta-20
Blocked agarose beads	bab-20
Spot-binding protein	etx-250
Spot-Tag peptide	ep-1
Spin columns	sct-10; sct-20; sct-50

Protocol for Immunoprecipitation of Spot-Tag-Fusion Proteins using Spot-Trap Agarose

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 re-suspending the cells. After washing:

Lyse cells

1. Re-suspend 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 supernatant. 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. Resuspend Spot-Trap Agarose beads and pipette 25 µl bead slurry into 500 µl ice-cold dilution buffer. Centrifuge at 2.500x g for 2 min at +4°C. Discard supernatant.

Bind proteins

5. Add diluted supernatant (step 3) to equilibrated Spot-Trap Agarose beads (step 4). If required, save 50 µl of diluted lysate for immunoblot analysis. Tumble end-over-end for 1 hour at 4°C.

Wash beads

6. Centrifuge at 2,500x g for 2 min at +4°C. If required, save 50 µl supernatant for immunoblot analysis. Discard remaining supernatant.
7. Re-suspend Spot-Trap Agarose beads in 500 µl ice-cold Spot Wash buffer. Rotate beads on a wheel for 5 min at 4°C. Centrifuge at 2.500x g for 2 min at +4°C. Discard supernatant and repeat wash twice. (change tube at last washing step).
   Note: It is recommended to wash 3x 5 min to obtain best results.

Elute proteins

8. Re-suspend Spot-Trap Agarose beads in 100 µl 2x SDS-sample buffer.
9. Boil re-suspended Spot-Trap Agarose beads for 10 min at 95°C to dissociate immunocomplexes from Spot-Trap Agarose beads. Beads can be separated by centrifugation at 2,500x g for 2 min at 4°C. Use supernatant for SDS-PAGE.
10. *Alternative elution options of Spot-Tag fusion proteins (instead of steps 8 and 9):*
    ***Peptide elution**: Reconstitute Spot-Tag peptide (ep-1) in PBS to a final concentration of 2 mg/ml (1.4 mM). Dilute Spot-Tag peptide to 100 µM in Dilution buffer and add 50-100 µl to Spot-Trap Agarose beads. Incubate for 10-30 min at room temperature or at 37 °C under slight agitation.*
    ***Alkaline elution**:* Re-suspend Spot-Trap Agarose beads in *50-100 µL 10 mM NaOH solution pH 12. Optional: Supplement with 500 mM NaCl for increased efficiency.* Pipette bead suspension up and down for at least 30 s.

    For either option: Centrifuge at 2,500x g for 2 min and carefully transfer supernatant to a new tube. To increase elution efficiency, this step can be repeated.
    For alkaline elution: adjust pH immediately after elution! Transfer the supernatant to a new tube and add 5 µl 0.2 M glycine pH 2.5 for neutralization.

    Note: For Western blot detection of Spot-Tag fusion proteins, use Spot-binding protein (etx-250) in conjunction with an anti-llama secondary antibody.
    Note: Use our spin column protocol for easy elution.

Support

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