

# Spot-Trap Agarose Kit 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)
Lysis buffer (ColP)		30 ml
RIPA buffer		30 ml
5x Dilution buffer		2 x 10 ml
5x Spot Wash buffer		2 x 10 ml

*Note: Add 40 ml H<sub>2</sub>O to 5x buffers before use. It is 5 times concentrated!*

*Note: 0.09 Na-Azide is added to buffers as an antiseptic and antifungal agent.*

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

## 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

### Buffer composition (as provided in the kit)

Buffer	Composition
Lysis buffer (ColP)	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

### Buffer composition (not provided in the kit)

Buffer	Composition
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

**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  $\sim 10^6 - 10^7$  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  $\mu$ l 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<sub>2</sub>, 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  $\mu$ l 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  $\mu$ l bead slurry into 500  $\mu$ l 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  $\mu$ l 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  $\mu$ l supernatant for immunoblot analysis. Discard remaining supernatant.
7. Re-suspend Spot-Trap Agarose beads in 500  $\mu$ l 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  $\mu$ l 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  $\mu$ M in Dilution buffer and add 50-100  $\mu$ l 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  $\mu$ l 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  $\mu$ l 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.

## Optional: Spin column protocol for immunoprecipitation and elution of proteins from Spot-Trap agarose beads

Note: spin columns (product codes sct-10, sct-20, sct-50) are not included

<b>Harvest cells</b>	<p>For one immunoprecipitation reaction the use of <math>\sim 10^6</math> - <math>10^7</math> 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:</p>
<b>Lyse cells</b>	<ol style="list-style-type: none"><li>1. Re-suspend cell pellet in 200 <math>\mu</math>l ice-cold lysis buffer by pipetting or using a syringe. <i>note: Supplement lysis buffer with protease inhibitors and 1 mM PMSF (not included).</i> <i>optional for nuclear/chromatin proteins: Use RIPA buffer supplemented with 1 mg/ml DNase, 2.5 mM MgCl<sub>2</sub>, protease inhibitors and 1 mM PMSF (not included).</i></li><li>2. Place the tube on ice for 30 min with extensively pipetting every 10 min.</li><li>3. Centrifuge cell lysate at 20.000x g for 10 min at +4°C. Transfer supernatant to a pre-cooled tube. Add 300 <math>\mu</math>l dilution buffer to supernatant. Discard pellet. <i>note: At this point cell lysate may be put at -80°C for long-term storage.</i> <i>optional: Add 1 mM PMSF and protease inhibitors (not included) to dilution buffer.</i></li></ol>
<b>Equilibrate beads</b>	<ol style="list-style-type: none"><li>4. Remove the upper cap of a new spin column and snap off the plug from the bottom of the spin column (Keep cap and plug!). Place the spin column in a 2 ml tube. Resuspend Spot-Trap Agarose beads and pipette 25 <math>\mu</math>l bead slurry into the spin column. Immediately add 500 <math>\mu</math>l ice-cold dilution buffer. Centrifuge at 100x g for 5-10 sec. Discard flow-through. Close column with the bottom plug.</li></ol>
<b>Bind proteins</b>	<ol style="list-style-type: none"><li>5. Add diluted supernatant (step 3) to equilibrated Spot-Trap Agarose beads (step 4). If required, save 50 <math>\mu</math>l of diluted lysate for immunoblot analysis. Secure the top of the spin column. Tumble end-over-end for 1 hour at 4°C.</li><li>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 <math>\mu</math>l flow-through for immunoblot analysis. Discard remaining flow-through.</li></ol>
<b>Wash beads</b>	<ol style="list-style-type: none"><li>7. Close spin columns with the bottom plug and place them in a 2 ml tube. Re-suspend Spot-Trap Agarose beads in 500 <math>\mu</math>l ice-cold Spot Wash buffer and rotate on a wheel for 5 min. Centrifuge at 100x g for 5-10 sec. Discard flow-through and repeat wash twice. (change tube at last washing step) <i>Note: It is recommended to wash 3x 5 min to obtain best results.</i></li></ol>
<b>Elute proteins</b>	<ol style="list-style-type: none"><li>8. Re-suspend Spot-Trap Agarose beads in 100 <math>\mu</math>l 2x SDS-sample buffer. Close column with the bottom plug.</li><li>9. Boil re-suspended Spot-Trap Agarose beads for 10 min at 95°C to dissociate immunocomplexes from Spot-Trap Agarose beads. Spot-Trap Agarose beads can be collected by centrifugation at 1000x g for 30-60s at 4°C and SDS-PAGE is performed with the eluate.</li><li>10. <i>Alternative elution options of Spot-Tag fusion proteins (instead of steps 8 and 9):</i> <b>Peptide elution:</b> 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 <math>\mu</math>M in Dilution buffer and add 50-100 <math>\mu</math>l to Spot-Trap Agarose beads. Incubate for 10-30 min at room temperature or at 37 °C under slight agitation. <b>Alkaline elution:</b> Re-suspend Spot-Trap Agarose beads in 50-100 <math>\mu</math>L 10 mM NaOH solution pH 12. <i>Optional: Supplement with 500 mM NaCl for increased efficiency.</i> 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. <b>For alkaline elution: adjust pH immediately after elution!</b> Transfer the supernatant to a new tube and add 5 <math>\mu</math>l 0.2 M glycine pH 2.5 for neutralization.</li></ol>
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