Spot-Trap Agarose Kit

Product code: etak-20

Introduction

The ChromoTek Spot-Trap® Agarose Kit consists of an anti-Spot Nanobody (VHH), which is covalently bound to agarose beads. Spot-Trap Agarose Kit is used to immunoprecipitate Spot-Tag® fusion proteins from cell extracts of various organisms like mammals, plants, bacteria, yeast, insects etc.

Properties

Ligand: Anti-Spot-tag single domain antibody fragment (VHH, Nanobody)
Reactivity: Specifically binds to Spot-tag sequence (PDRVRAVSHWSS). Compatible with N- and C-terminal tagging, internal tagging must be tested from case by case.
Binding capacity: 17.5 µg of recombinant Spot-tagged protein (~30 kDa) per 25 µL bead slurry
Bead size: 90 µm (cross-linked 4 % agarose beads)
Buffer compatibility: See *Wash buffer compatibility table*.
Storage buffer: 20 % ethanol
Storage conditions: Upon receipt store at +4°C. Do not freeze!
Stability: Stable for 1 year upon receipt.
Shipment: Shipped at ambient temperature.
RRID: AB_2827590

Suggested buffer compositions

Buffers provided in the kit

Buffer	Composition	Quanitity
Lysis buffer	10 mM Tris/Cl pH 7.5, 150 mM NaCl, 0.5 mM EDTA, 0.5 % Nonidet™ P40 Substitute, 0.09 % sodium azide	30 mL
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 % sodium azide	30 mL
Dilution buffer*	10 mM Tris/Cl pH 7.5, 150 mM NaCl, 0.5 mM EDTA, 0.018 % sodium azide	50 mL (after dilution with 40 mL H_2O)
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 % sodium azide	50 mL (after dilution with 40 mL H_2O)

*Add 40 mL H_2O to Dilution buffer and Wash buffer before use. The indicated buffer composition refers to the diluted buffer solution.

Note: Sodium azide is added to buffers as antiseptic and antifungal agent.
Note: Use your equivalent cell lysis buffer for other cell types like yeast, plants, insects, bacteria.

Required buffer solutions

Buffer	Composition
2x SDS-sample buffer	120 mM Tris/Cl pH 6.8, 20 % glycerol, 4 % SDS, 0.04 % bromophenol blue, 10 % β-mercaptoethanol
Alkaline elution buffer	10 mM NaOH pH 12 (optional: supplemented with 500 mM NaCl)
Neutralization buffer	200 mM glycine pH 2.5 (adjust the pH at +4°C)

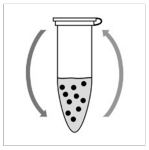
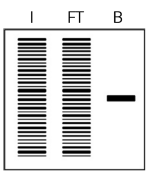
Wash buffer compatibility table

Buffer ingredients	Max. concentration
Deoxycholate	1 %
DTT	10 mM
Guanidine HCl	750 mM
NaCl	2 M
Nonidet™ P40 Substitute	tested up to 2 %
SDS	0.1 %
Triton™ X-100	tested up to 1 %
Urea	2 M

Product sizes

Product	Product code	Size
Spot-Trap® Agarose	eta-10	10 reactions (250 µL slurry)
	eta-20	20 reactions (500 µL slurry)
	eta-100	100 reactions (2.5 mL slurry)
	eta-200	200 reactions (5 mL slurry)
	eta-400	400 reactions (10 mL slurry)
Spot-Trap® Agarose Kit	etak-20	20 reactions (500 µL slurry) including buffers

Protocol at a glance

General		• Perform all steps at +4°. • Use your preferred cell lysis buffer and cell lysis conditions.
Cell Lysis		• Use 10^6-10^7 cells and 200 µL Lysis buffer. • Perform cell lysis and clear lysate. • Mix 200 µL cleared lysate with 300 µL Dilution buffer.
Bead equilibration		• Transfer 25 µL bead slurry into a 1.5 mL tube. • Equilibrate beads 3x with 500 µL Dilution Buffer.
Protein binding		• Add 500 µL diluted lysate to beads. • Rotate end-over-end for 1 hour at +4°C.
Washing		• Wash beads 3x with 500 µL Wash buffer. • Transfer beads to a new tube during the last washing step.
Elution with SDS-sample buffer		• Resuspend beads in 80 µL 2x SDS-sample buffer. • Boil beads for 5 min at +95°C. • Analyze the supernatant in SDS-PAGE / Western Blot.

Immunoprecipitation protocol

Cell material

The following protocol describes the preparation of mammalian cell lysate!
For other type of cells, we recommend using 500 µg of cell extract and start the protocol with step *Bead equilibration*.

Mammalian cell lysis

Note: Harvesting of cells and cell lysis should be performed with ice-cold buffers. We strongly recommend to add protease inhibitors to the Lysis buffer to prevent degradation of your target protein and its binding partners.
For one immunoprecipitation reaction, we recommend using ~10^6-10^7 cells.

1. Choice of lysis buffer:
   - For cytoplasmic proteins, resuspend the cell pellet in 200 µL ice-cold Lysis buffer by pipetting up and down. Supplement Lysis buffer with protease inhibitor cocktail and 1 mM PMSF (not included).
   - For nuclear/chromatin proteins, resuspend cell pellet in 200 µL ice-cold RIPA buffer supplemented with DNaseI (f.c. 75-150 Kunitz U/mL), $MgCl_2$ (f.c. 2.5 mM), protease inhibitor cocktail and PMSF (f.c. 1 mM) (not included).
2. Place the tube on ice for 30 min and extensively pipette the suspension every 10 min.
3. Centrifuge cell lysate at 17,000x g for 10 min at +4°C. Transfer cleared lysate (supernatant) to a pre-cooled tube and add 300 µL Dilution buffer supplemented with 1 mM PMSF and protease inhibitor cocktail (not included). If required, save 50 µL of diluted lysate for further analysis (input fraction).

Bead equilibration

1. Resuspend the beads by gently pipetting up and down or by inverting the tube. Do not vortex the beads!
2. Transfer 25 µL of bead slurry into a 1.5 mL reaction tube.
3. Add 500 µL ice-cold Dilution buffer.
4. Sediment the beads by centrifugation at 2,500x g for 5 min at +4°C. Discard the supernatant.

Note: Alternatively, Spin columns (sct-10; -20; -50) can be used to equilibrate the beads.

Protein binding

1. Add diluted lysate to the equilibrated beads.
2. Rotate end-over-end for 1 hour at +4°C.

Washing

1. Sediment the beads by centrifugation at 2,500x g for 5 min at +4°C.
2. If required, save 50 µL of supernatant for further analysis (flow-through/non-bound fraction).
3. Discard remaining supernatant.
4. Resuspend beads in 500 µL Wash buffer.
5. Rotate end-over-end for 5 min at +4°C.
6. Sediment the beads by centrifugation at 2,500x g for 5 min at +4°C. Discard remaining supernatant.
7. Repeat this step at least twice.
8. During the last washing step, transfer the beads to a new tube.

Optional: To increase stringency of the Wash buffer, test various salt concentrations e.g. 150-500 mM, and/or add a non-ionic detergent e.g. Triton™ X-100 (see *Wash buffer compatibility table* for maximal concentrations).
Note: Alternatively, Spin columns (sct-10; -20; -50) can be used to wash the beads.

Elution with 2x SDS-sample buffer (Laemmli)

1. Remove the remaining supernatant.
2. Resuspend beads in 80 µL 2x SDS-sample buffer.
3. Boil beads for 5 min at +95°C to dissociate immunocomplexes from beads.
4. Sediment the beads by centrifugation at 2,500x g for 2 min at +4°C.
5. Analyze the supernatant in SDS-PAGE / Western Blot.

Note: For Western blot detection we recommend Spot VHH (etb-250) in conjunction with a secondary antibody or Spot-Label (eba488 or eba594).

Elution with Alkaline elution buffer

1. Remove the remaining supernatant.
2. Add 50–100 µL Alkaline elution buffer and constantly pipette up and down for 30-60 sec at +4°C or room temperature.
3. Sediment the beads by centrifugation at 2,500x g for 5 min at +4°C.
4. Transfer the supernatant to a new tube.
5. Immediately neutralize the eluate fraction with Neutralization buffer.
6. Repeat this step at least once to increase elution efficiency.

Note: Elution at room temperature is more efficient than elution at +4°C. Prewarm buffers for elution at room temperature.
Note: Alternatively, Spin columns (sct-10; -20; -50) can be used to separate the beads.
Optional: Spot-tagged fusion proteins can be eluted with Spot-peptide at room temperature. For protein purification and efficient elution with Spot-peptide at +4°C we recommend Spot-Cap® affinity resin.

Product overview and related products

Spot-tag toolbox	Product code
Spot-Trap® Agarose	eta-10; -20; -100
Spot-Trap® Agarose Kit	etak-20
Spot-Trap® Magnetic Agarose	etma-10; -20; -100
Spot-Trap® Magnetic Agarose Kit	etmak-20
Spot-Trap® Dynabeads	etd-10; -20; -100
Spot-Trap® Dynabeads Kit	etdk-20
iST Spot-Trap® Kit for IP/MS	etak-iST-8
Binding Control Agarose	bab-20
Binding Control Magnetic Agarose	bmab-20
Spin columns	sct-10; sct-20; sct-50
Spot-Label® ATTO488 Spot-Label® ATTO594	eba594-10; -50 eba647n-10; -50
Spot VHH, recombinant binding protein (bivalent)	etb-250
Spot-Cap®	eca-2
Spot-peptide	ep-1; -10
Spot-Cap® and peptide	eca-ep
Spot Vectors for cloning: pSpot1 vector, E. coli, Spot-tag N-term., Kan., high expression pSpot2 vector, E. coli, Spot-tag C-term., Kan., high expression pSpot3 vector, E. coli, Spot-tag C-term., Amp., low expression pSpot4 vector, E. coli, Spot-tag N-term., Amp., low expression pSpot5 vector, S. cerevisiae, Spot-tag N-term., Leu, CEN, low expression pSpot6 vector, S. cerevisiae, Spot-tag C-term., Leu, CEN, low expression pSpot7 vector, S. cerevisiae, Spot-tag N-term., Leu, 2µ, high expression pSpot8 vector, S. cerevisiae, Spot-tag C-term., Leu, 2µ, high expression	 ev-1 ev-2 ev-3 ev-4 ev-5 ev-6 ev-7 ev-8
Spot Vectors - positive controls: pSpot-Tag-Actin vector (plasmid) for expression in mammalian cells pSpot2_GFPSpot-Tag vector (plasmid) for expression in E. coli pSpot8_GFP-Spot-Tag vector (plasmid) for expression in S. cerevisiae	 ev-31 ev-32 ev-33

For product details, information, and ordering visit www.chromotek.com.

Contact

support@chromotek.com

ChromoTek GmbH
Am Klopferspitz 19
82152 Planegg-Martinsried
Germany
phone: +49 89 124 148 80
fax: +49 89 124 148 811

ChromoTek Inc.
62-64 Enter Lane
Islandia, NY 11749
USA
phone: 631 501 1058
fax: 631 501 1060

Disclaimer

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