Spot-Cap™

Product code: eca

Introduction

The ChromoTek Spot-Cap™ consists of an anti-Spot-tag® Nanobody (VHH), which is covalently bound to agarose. Spot-Cap is used to purify Spot-tagged proteins from cell extracts of various organisms like mammals, plants, bacteria, yeast, insects etc. Spot-Cap is engineered for gentle elution with Spot-peptide at 4°C and high yields in batch or gravity flow formats.

Properties

Binding capacity (static)	10 mg protein (30 kDa) / 1 mL settled resin (340 nmol / 1 mL settled resin)
Elution conditions (at +4°C)	0.1 mM Spot-peptide or 100 mM glycine pH 2.0
Regeneration buffer	100 mM glycine pH 2.0
Regeneration	>5 cycles
Purification format	Batch and gravity flow
Form	50% slurry
Matrix, particle size	4% agarose, 90 µm
pH range for purification	pH 4 - 11
Chemical stability	1 M NaCl, 2 M Urea, 10 mM DTT, 10 mM ß-mercaptoethanol, 10 mM TCEP, 2% DDM, 2% Nonidet™ P40, 2% Triton™ X-100
Storage buffer	20% EtOH
Storage conditions	Spot-Cap: Upon receipt store at +4°C. Do not freeze! Spot-peptide: Upon receipt store at -20°C.
Stability	Stable for 1 year upon receipt.
Shipment	Shipped at ambient temperature.

Suggested buffer compositions

Buffer	Composition
Lysis, binding and wash buffer	commonly used buffers with pH range 4 - 11 (see *Buffer compatibility table* for special buffer additives)
Elution with Spot-peptide	100 µM Spot-peptide in PBS
Glycine elution	100 mM glycine pH 2.0
Neutralization	1 M Tris pH 10.4 (adjust pH at +4°C)
Regeneration	100 mM glycine pH 2.0
Storage	20% ethanol

Purification protocol

General Remarks

- Harvesting of cells and cell lysis should be performed with ice-cold buffers.
- Supplement lysis buffer with protease inhibitors (e.g. 1 mM PMSF), DNase I (final concentration 75-150 Kunitz U/mL) and $MgCl_2$ (final concentration 2.5 mM) and if necessary, with phosphatase inhibitors.
- Centrifuge and/or filter through a filter (0.25 µm) before applying the cell lysate to the agarose resin.
- Use a large pipette (cut tip if necessary) to pipette Spot-Cap resin.
- Spot-Cap is optimized for protein purification at +4°C. Purification at room temperature is also possible but needs to be tested for the particular Spot-tagged protein.

Batch purification

Resin equilibration

1. Resuspend Spot-Cap resin by gently pipetting up and down. Do not vortex the beads!
2. Transfer the desired volume of slurry to an appropriate tube.
3. Sediment beads by centrifugation at 2,500 × g for 2-5 minutes. Carefully remove the supernatant (storage solution) and discard it.
4. Add 10 bed volumes (BV) of lysis buffer to equilibrate the beads. Invert to mix.
5. Sediment beads by centrifugation at 2.500 x g for 2-5 minutes. Carefully remove the supernatant and discard it.
6. Repeat this step twice.

Protein binding

1. Add clarified lysate to the equilibrated beads.
2. Close the tube and incubate for 30-60 minutes at +4°C with gentle mixing (e.g. end-over-end rotation).

Note: The binding efficiency may differ significantly between different Spot-tagged proteins.

Washing

1. Sediment beads by centrifugation at 2,500 × g for 2-5 minutes. Carefully remove the supernatant and discard it.
2. Wash beads with 10-20 BVs of ice-cold wash buffer. Invert to mix.
3. Sediment beads by centrifugation at 2,500 x g for 2-5 minutes. Carefully remove the supernatant and discard it.
4. Repeat this step twice.
5. During the last washing step, transfer the beads to a new tube.

Note: Volumes and times used may vary from protein to protein.

Optional: To increase stringency of the wash buffer, test various salt concentrations e.g. 150 mM – 500 mM, and/or add a non-ionic detergent e.g. Nonidet™ P40 or Triton™ X-100 (see *Buffer compatibility table* for maximal concentrations).

Elution with Spot-peptide

1. Prepare Spot-peptide stock solution by dissolving lyophilized Spot-peptide (ep-1 or ep-10) as described in the datasheet.
2. Dilute Spot-peptide to a concentration of 100 µM in PBS buffer.
3. Remove the remaining supernatant from the beads.
4. Add 2 BVs diluted Spot-peptide and mix well. Incubate for 5-10 min.
5. Sediment beads by centrifugation at 2,500 x g for 2-5 minutes.
6. Transfer the elate fraction to a new tube.
7. Repeat this step 1-6 times to increase elution efficiency.

Optional: Spot-peptide can also be dissolved in other commonly used buffers.
Optional: Use 500 µM Spot-peptide for faster elution.
Note: Aliquot the stock solution of Spot-peptide and store at -20°C. Always freshly prepare the diluted Spot-peptide solution (100 µM) from the stock.
Note: Some Spot-tagged protein may remain bound to the beads. Volumes and times used for elution may vary among proteins. Additional elution steps may be required.

Acidic elution with glycine elution buffer

1. Remove the remaining supernatant.
2. Add 1-2 BVs glycine elution buffer and constantly pipette up and down for 30-60 sec.
3. Sediment beads by centrifugation at 2,500 x g for 2-5 minutes.
4. Immediately neutralize the eluate fraction with neutralization buffer.
5. Repeat this step several times to increase elution efficiency.

Note: Some Spot-tagged protein may remain bound to the beads. Volumes and times used for elution may vary among proteins. Additional elution steps may be required.

Gravity Flow column purification

Resin equilibration

1. Resuspend Spot-Cap resin by gently pipetting up and down. Do not vortex the beads!
2. Transfer the desired volume of bead slurry to gravity flow column (e.g. Poly-Prep® Chromatography Columns, Bio-Rad catalogue no. 7311550).
3. Allow the beads to drain by gravity flow.
4. Add 10 column volumes (CV) of lysis buffer to equilibrate the beads and allow the column to drain by gravity flow.

Protein binding

1. Add clarified lysate to the equilibrated beads.
2. Close the column and incubate for 15-60 minutes at +4°C with gentle mixing (e.g. end-over-end rotation).

Optional: To ensure quantitative binding of the Spot-tagged protein, the flow through may be added to the beads again and the above steps may be repeated.
Note: The binding efficiency may differ significantly between different Spot-tagged proteins.

Washing

1. Allow the beads to drain by gravity flow and collect flow through.
2. Wash beads with 10-20 CVs of ice-cold wash buffer.
3. Allow the column to drain by gravity flow.
4. Repeat this step twice.

Note: Volumes and times used may vary from protein to protein.

Optional: To increase stringency of the wash buffer, test various salt concentrations e.g. 150 mM – 500 mM, and/or add a non-ionic detergent e.g. Nonidet™ P40 or Triton™ X-100 (see *Buffer compatibility table* for maximal concentrations).

Elution with Spot-peptide

1. Prepare Spot-peptide stock solution by dissolving lyophilized Spot-peptide (ep-1 or ep-10) as described in the datasheet.
2. Dilute Spot-peptide to a concentration of 100 µM in PBS buffer.
3. Allow the column to drain by gravity flow.
4. Add 2 CVs diluted Spot-peptide and close the column. Incubate for 5-10 min with gentle mixing (e.g. end-over-end rotation).
5. Allow the column to drain by gravity flow and collect the eluate fraction.
6. Repeat this step 1-6 times to increase elution efficiency.

Optional: Spot-peptide can also be dissolved in other commonly used buffers.

Optional: Use 500 µM Spot-peptide for faster elution.

Note: Aliquot the stock solution of Spot-peptide and store at -20°C. Always freshly prepare the diluted Spot-peptide solution (100 µM) from the stock.

Note: Some Spot-tagged protein may remain bound to the beads. Volumes and times used for elution may vary among proteins. Additional elution steps may be required.

Acidic elution with glycine elution buffer

1. Allow the column to drain by gravity flow.
2. Add 1-2 CVs glycine elution buffer.
3. Allow the column to drain by gravity flow.
4. Immediately neutralize the eluate fraction with neutralization buffer.
5. Repeat this step several times to increase elution efficiency.

Note: Some Spot-tagged protein may remain bound to the beads. Volumes and times used for elution may vary among proteins. Additional elution steps may be required.

Regeneration

1. Wash beads twice with 10-20 BVs/CVs regeneration buffer.
2. Wash beads twice with 10-20 BVs/CVs wash buffer.
3. Wash beads with 10-20 BVs/CVs 20% ethanol.
4. Store beads in 20% ethanol at +4°C.

Optional: Spot-Cap regeneration can be performed at ambient temperature.
Note: Spot-Cap can be regenerated at least 5 times with minimal loss of binding capacity.
Note: The reuse of Spot-Cap depends on the nature of the sample and should only be performed with identical samples to prevent cross-contamination.
Note: Do not leave Spot-Cap in regeneration buffer more than 30 minutes.

Buffer compatibility table

Buffer ingredients	Tested up to
ß-mercaptoethanol	10 mM
DDM	2%
DTT	10 mM
NaCl	1 M
Nonidet™ P40 Substitute	2 %
SDS	0.1 % during binding (0% during washing)
TCEP	10 mM
TritonTM X-100	2 %
Urea	2 M

Product sizes

Product	Product Code	Size
Spot-Cap™	eca-2	2 mL slurry
Spot-Peptide	ep-1; ep-10	1 mg; 10 mg
Spot-Cap™ and Peptide	eca-ep	0.1 mL Spot-Cap™ + 1 mg Spot-Peptide

Related Products

Spot system	Code
Spot-Cap™	eca-2
Spot-Cap™ and Peptide	eca-ep
Spot-peptide	ep-1; -10
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-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
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 VHH, recombinant binding protein	etb-250
Spot-Label® ATTO488 Spot-Label® ATTO594	eba488-10; -50 eba594-10; -50

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

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