

# Myc-Trap<sup>®</sup>\_A for immunoprecipitation of Myc-tagged 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 Myc epitope tag is widely used to detect expression of recombinant proteins in bacteria, yeast, insect and mammalian cell systems. For biochemical analysis including mass spectrometry and enzyme activity measurements these Myc tagged proteins and their interacting factors can be isolated fast and efficiently by immunoprecipitation using the Myc-Trap<sup>®</sup>. Myc-Trap<sup>®</sup> utilizes small recombinant antibody fragments covalently coupled to the surface of agarose beads.

## Specificity

Myc-Trap recognizes Myc-Tag sequence EQKLISEEDL at the N-terminus, C-terminus, or internal site of the fusion protein.

## Content

Reagent	Code	Quantity
Myc-Trap <sup>®</sup> _A	yta-20	20 reactions (0.5 ml slurry)
Myc -Trap <sup>®</sup> _A	yta -100	100 reactions (2.5 ml slurry)
Myc -Trap <sup>®</sup> _A	yta -200	200 reactions (5 ml slurry)

## 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
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
Dilution/Wash buffer	10 mM Tris/Cl pH 7.5; 150 mM NaCl; 0.5 mM EDTA
Myc peptide	Reconstitute Myc peptide (code yp-1) in water to a final concentration of 2 mg/ml.
2x Myc peptide	Reconstitute Myc peptide (code 2yp-1) in PBS to a final concentration of 1 mg/ml.
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

Myc Toolbox	Code
Myc -Trap <sup>®</sup> _A Kit	ytak-20
Blocked agarose beads	bab-20
Myc-tag antibody [9E1]	9e1
Myc peptide	yp-1
2x Myc peptide	2yp-1
Spin columns	sct-10; sct-20; sct-50

## Support

Please refer to our FAQ section at [www.chromotek.com](http://www.chromotek.com) or contact [support@chromotek.com](mailto:support@chromotek.com)

## 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 resuspending the cells. After washing:

## Lyse cells

1. Resuspend 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. Vortex Myc-Trap®\_A 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 and repeat wash twice.

## Bind proteins

5. Add diluted supernatant (step 3) to equilibrated Myc-Trap®\_A 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.
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. Resuspend Myc-Trap®\_A beads in 500  $\mu$ l ice-cold dilution buffer. Centrifuge at 2.500x g for 2 min at +4°C. Discard supernatant and repeat wash twice.

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

## Wash beads

8. Resuspend Myc-Trap®\_A beads in 100  $\mu$ l 2x SDS-sample buffer.
9. Boil resuspended Myc-Trap®\_A beads for 10 min at 95°C to dissociate immunocomplexes from Myc-Trap®\_A beads. Myc-Trap®\_A beads can be collected by centrifugation at 2.500x g for 2 min at 4°C and SDS-PAGE is performed with the supernatant.

## Elute proteins

10. *Optional instead of steps 8 and 9:*

**For elution of 1xMyc-tagged fusion proteins:** Reconstitute Myc peptide (code yp-1) in water to a final concentration of 2 mg/ml.

**For elution of 2x Myc-tagged fusion proteins:** Reconstitute 2x Myc peptide (code 2yp-1) in PBS to a final concentration of 1 mg/ml.

*Dilute Myc peptide to 50  $\mu$ g/ml (2x Myc peptide: 100  $\mu$ g/ml) in Dilution buffer and add 50-100  $\mu$ l to Myc-Trap®\_A beads. Mix for 15 min at RT. Centrifuge at 2500x g for 2 min and carefully transfer supernatant to a new tube. To increase elution efficiency this step can be repeated.*

*Note: Use our spin column protocol for easy elution*

# Optional: Spin column protocol for immunoprecipitation and elution of proteins from Myc-Trap<sup>®</sup> agarose beads

Note: spin columns (product code sct-10) are not included

<b>Harvest cells</b>	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 resuspending the cells. After washing:
<b>Lyse cells</b>	<ol style="list-style-type: none"><li>1. Resuspend 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. Vortex Myc-Trap<sup>®</sup>_A 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 and repeat wash twice. 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 Myc-Trap<sup>®</sup>_A 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. Resuspend Myc-Trap<sup>®</sup>_A beads in 500 <math>\mu</math>l 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. <i>optional: Increase salt concentration in the second washing step up to 500 mM.</i></li></ol>
<b>Elute proteins</b>	<ol style="list-style-type: none"><li>8. <b>For elution of 1xMyc-tagged fusion proteins:</b> Reconstitute Myc peptide (code yp-1) in water to a final concentration of 2 mg/ml. <b>For elution of 2x Myc-tagged fusion proteins:</b> Reconstitute 2x Myc peptide (code 2yp-1) in PBS to a final concentration of 1 mg/ml. <i>Dilute Myc peptide to 50 <math>\mu</math>g/ml (2x Myc peptide: 100 <math>\mu</math>g/ml) in Dilution buffer and add 50-100 <math>\mu</math>l to Myc-Trap<sup>®</sup>_A beads. Mix for 15 min at RT. Remove bottom plug of the spin column and place it in a new tube. Centrifuge at 1000x g for 30-60 sec. To increase elution efficiency this step can be repeated.</i></li></ol>

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