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Myc-Trap®_A Kit 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®. Myc-Trap® 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®_A kit	ytak-20	20 reactions (0.5 ml resin)
Lysis buffer (CoIP)		30 ml
RIPA buffer		30 ml
5x Wash / Dilution buffer		2 x 10 ml
Elution buffer		3 x 1 ml

Note: Add 40 ml H_2O to 5x Wash/ Dilution buffer before use. It is 5 times concentrated!!
Note: 0,09 Na-Azide is added to our 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 (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/Wash buffer	10 mM Tris/Cl pH 7.5; 150 mM NaCl; 0.5 mM EDTA, 0,018% Na-Azide

Buffer composition (not provided in the kit)

Buffer	Composition
Myc peptide	reconstitute Myc peptide (code yp-1) in water to a final concentration of 2 mg/ ml
2x Myc peptide	reconstitute 2x 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®_A	yta-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

Protocol for Immunoprecipitation of Myc-Fusion Proteins using Myc-Trap®_A

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 resuspending the cells. After washing:

Lyse cells

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

Bind proteins

5. Add diluted supernatant (step 3) to equilibrated Myc-Trap®_A beads (step 4). If required, save 50 µl 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 µl supernatant for immunoblot analysis. Discard remaining supernatant.

Wash beads

7. Resuspend Myc-Trap®_A beads in 500 µl 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.

Elute proteins

8. Resuspend Myc-Trap®_A beads in 100 µl 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.
10. *Optional instead of steps 8 and 9:*

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

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

    Dilute Myc peptide to 50 µg/ml (2x Myc peptide: 100 µg/ml) in Dilution buffer and add 50-100 µl 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.

Support

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

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

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

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 resuspending the cells. After washing:

Lyse cells

1. Resuspend 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. 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®_A beads and pipette 25 µl bead slurry into the spin column. Immediately add 500 µl 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.

Bind proteins

5. Add diluted supernatant (step 3) to equilibrated Myc-Trap®_A beads (step 4). If required, save 50 µl of diluted lysate for immunoblot analysis. Secure the top of the spin column. Tumble end-over-end for 1 hour at 4°C.
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 µl flow-through for immunoblot analysis. Discard remaining flow-through.

Wash beads

7. Resuspend Myc-Trap®_A beads in 500 µl 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.

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

Elute proteins

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

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

   Dilute Myc peptide to 50 µg/ml (2x Myc peptide: 100 µg/ml) in Dilution buffer and add 50-100 µl to Myc-Trap®_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.

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