MBP-Trap_A for Immunoprecipitation of MBP-Fusion Proteins

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

Introduction

Fusion of proteins to *E.coli* Maltose binding protein (MBP) is a common technique to increase solubility and expression level of a protein. Furthermore, MBP- tagged fusion proteins are often used in protein-protein interaction studies. Both approaches require highly specific tools to isolate MBP fusion proteins. MBP-fusion proteins and their interacting factors can be isolated fast and efficiently by immunoprecipitation using the MBP-Trap. MBP-Trap utilizes small recombinant alpaca antibody fragments covalently coupled to the surface of agarose beads.

Specificity

E.coli Maltose binding protein

Content

Reagent	Code	Quantity
MBP-Trap_A	mbta-20	20 reactions (0.5 ml slurry)
MBP-Trap_A	mbta-100	100 reactions (2.5 ml slurry)
MBP-Trap_A	mbta-200	200 reactions (5 ml slurry)
MBP-Trap_A	mbta-400	400 reactions (10 ml slurry)

Bead properties

Bead size: ~ 90 µm (cross-linked 4% agarose beads)
Storage buffer: 20% EtOH
Binding capacity: 10 µl MBP-Trap_A slurry binds 0,25 nmol MBP. Binding capacity was determined under optimal conditions with recombinant MBP-fusion protein (74,2 kDa) and will vary depending on the MBP-fusion protein and assay conditions.

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
Dilution/Wash buffer	10 mM Tris/Cl pH 7.5; 150 mM NaCl; 0.5 mM EDTA
Glycine-elution buffer	200 mM glycine pH 2.5
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

MBP Toolbox	Code
MBP-binding protein	mbt-250
MBP-Trap_A Kit	mbtak-20
Binding control agarose beads	bab-20
Spin columns	sct-10; sct-20; sct-50

Support

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

Protocol for Immunoprecipitation of MBP-Fusion Proteins using MBP-Trap_A

We recommend to use 0.5 – 1 mg of protein extract or 1 – 5 µg recombinant protein per Immunoprecipitation.

Equilibrate beads

1. Vortex MBP-Trap_A beads and pipette 25 µl bead slurry into 500 µl ice-cold wash/dilution buffer. Centrifuge at 2.500x g for 2 min at +4°C. Discard supernatant and repeat wash twice.

Bind proteins

2. Add protein extract or recombinant MBP protein diluted in wash/dilution buffer to equilibrated MBP-Trap_A beads (step 1). If required, save input for immunoblot analysis. Tumble end-over-end for 1 hour at 4°C.

Wash beads

3. Centrifuge at 2.500x g for 2 min at +4°C. If required, save supernatant for immunoblot analysis. Discard remaining supernatant.
4. Resuspend MBP-Trap_A beads in 500 µl ice-cold wash/dilution buffer.
5. 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

6. Resuspend MBP-Trap_A beads in 100 µl 2x SDS-sample buffer.
7. Boil resuspended MBP-Trap_A beads for 10 min at 95°C to dissociate immunocomplexes from MBP-Trap_A beads. MBP-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.
8. *optional instead of steps 8 and 9: elute bound proteins by adding 50 µl 0.2 M glycine pH 2.5 (incubation time: 30 sec under constant mixing) followed by centrifugation. Transfer supernatant to a new tube and add 5 µl 1M Tris base pH 10.4 for neutralization. To increase elution efficiency this step can be repeated.*
   Note: Efficiency of glycine elution will vary depending on MBP-fusion protein. Glycine elution is not as efficient as SDS sample buffer elution.