Datasheet for ABIN5311504

MBP-Catcher

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Overview

Quantity:	2000 µL
Target:	Maltose Binding Protein (MBP)
Reactivity:	E. coli
Expression System:	E.coli
Application:	Protein Complex Immunoprecipitation (Co-IP), Immunoprecipitation (IP), Purification (Purif), Chromatin Immunoprecipitation (ChIP), RNA-Binding Protein Immunoprecipitation (RIP)

Product Details

Sample Type:	Cell Extracts
Specificity:	The Antibody with Clone 1G5 recognizes E.coli maltose-binding protein (MBP)
Characteristics:	MBP-Catcher is based on a high-affinity single-domain antibody (sdAb) that is covalently immobilized on 4 % cross-linked agarose beads. The innovative, oriented and selective attachment via a flexible linker guarantees a high accessibility of the sdAbs and largely eliminates batch-to-batch variations. Due to the single-chain nature of sdAbs and their covalent attachment, no "leakage" of light and heavy chains from IgGs is observed during elution with SDS sample buffer. MBP-Catcher thus features high affinity and superior capacity for MBP fusion proteins while showing negligible non-specific background. MBP-Catcher is compatible not only with physiological buffers but also with high stringency buffers. MBP-Catcher thus provides great freedom to adjust the binding and washing conditions to the experimental needs.
Components:	4 % cross-linked agarose (bead size 50-150 µm) with covalently immobilized single-domain antibody

Product Details

Material not included:	wash buffers, columns, tubes
Bead Ligand:	Antibody
Bead Matrix:	Agarose beads
Bead Size:	90 µm

Target Details

Target:	Maltose Binding Protein (MBP)
Alternative Name:	maltose binding protein, MBP (MBP Products)
Background:	Maltose-binding protein (MBP) is encoded by the malE gene from the gram-negative bacterium Escherichia coli. When expressed as a fusion protein it boosts the expression of the fusion partner and is therefore a common entity in bacterial expression vectors

Application Details

Application Notes:	200 µL slurry for up to 10 reactions
Comment:	Capacity: > 2.5 µg MBP per µl of packed beads (i.e. 2 µl of slurry)
Protocol:	This protocol provides a general outline of how to use MBP-Catcher (agarose beads) for immunoprecipitation using a microcentrifuge for sedimentation. Alternatively, it is possible to use MBP-Catcher agarose beads in spin columns. All protocol steps should be carried out at 4 °C. Protocol as PDF 1. For mammalian cells, harvest 10^6-10^8 cells per sample. 2. Lyse cells according to established protocols in 0.2 to 1.5 mL volume. Recommended Buffer Conditions: MBP-Catcher resins are compatible with commonly used Lysis and Washing buffers, e.g. RIPA buffer. The following buffer conditions have been tested: • pH ranging from pH 5 to pH 9 • 2 % Triton X-100, 1 % Tween-20, 1 % NP-40, 1 % CHAPS, 1 % Deoxycholate, 0.1 % SDS • 4 M NaCl, 2 M KCl, 1 M $MgCl_2$ • 100 mM EDTA • 4 M urea • 10 mM DTT, 10 mM 2-Mercaptoethanol • Protease Inhibitors • RNAse A, DNAse I, Benzonase 3. Centrifuge cell lysates in microcentrifuge tubes for 10 min at 14.000 x g at 4 °C. Keep a small samples as “input” fraction. 4. Transfer the supernatant to a fresh microcentrifuge tube for each sample and keep at 4 °C.

Application Details

5. Homogenize the MBP-Catcher (agarose beads) slurry gently by shaking.
6. Transfer 20 µL bead slurry to a 1.5 mL microcentrifuge tube for each sample.
7. Add 1 mL Lysis Buffer to equilibrate MBP-Catcher (agarose beads).
8. Centrifuge MBP-Catcher (agarose beads) for 1 min at 1000 x g and carefully remove the supernatant.
9. Repeat wash steps once for a total of two washes.
10. Resuspend equilibrated MBP-Catcher (agarose beads) gently with the cell lysate supernatant.
11. Rotate the microcentrifuge tubes for 1 h at 4 °C.
12. Centrifuge microcentrifuge tubes for 1 min at 1000 x g at 4 °C. Keep a small sample as “unbound” fraction. Carefully remove the supernatant.
13. Resuspend MBP-Catcher (agarose beads) in 1 mL Lysis Buffer.
14. Centrifuge MBP-Catcher (agarose beads) for 1 min at 1000 x g and carefully remove the supernatant.
15. Repeat wash steps twice for a total of three washes.
16. Resuspend MBP-Catcher (agarose beads) gently in 1 mL TBS.
17. Centrifuge MBP-Catcher (agarose beads) for 1 min at 1000 x g and carefully remove the supernatant.
18. Resuspend MBP-Catcher (agarose beads) gently in 1 mL TBS.
19. Centrifuge MBP-Catcher (agarose beads) for 1 min at 3000 x g and carefully remove the supernatant.
20. Resuspend MBP-Catcher (agarose beads) resin in 50 µL 2X SDS samples buffer.
21. Heat MBP-Catcher (agarose beads) resin for 5 min to 95 °C.
22. Centrifuge microcentrifuge tubes for 1 min at 3000 x g and transfer the supernatant to fresh microcentrifuge tubes. Keep the MBP-Catcher (agarose beads) as backup.

Restrictions:	For Research Use only

Handling

Buffer:	50 % slurry in 20 % ethanol / PBS 0.09 % sodium azide
Preservative:	Sodium azide
Precaution of Use:	This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
Storage:	4 °C