Datasheet for ABIN7529450

RFP-Catcher (agarose magnetic beads)

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Overview

Quantity:	2000 µL
Target:	RFP
Reactivity:	Discosoma
Application:	RNA-Binding Protein Immunoprecipitation (RIP), Protein Complex Immunoprecipitation (Co-IP), Immunoprecipitation (IP), Purification (Purif), Chromatin Immunoprecipitation (ChIP)

Product Details

Purpose:	RFP-Catcher is based on a high-affinity single-domain antibody (sdAb) that is covalently immobilized on 4% cross-linked magnetic agarose.
Specificity:	MRFP (red fuorescent protein) and other derivatives like mOrange, dsRed1, dsRED2, tdTomato, mCherry and mScarlet-i.
Cross-Reactivity (Details):	Does not cross-react with GFP or mTagBFP derivatives.
Characteristics:	RFP-Catcher is based on a high-affinity single-domain antibody (sdAb) that is covalently immobilized on 4 % cross-linked magnetic 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. RFP-Catcher thus features high affinity and superior capacity for RFP fusion proteins while showing negligible non-specific background. RFP-Catcher immobilizes a wide range of RFP derivatives. RFP-Catcher is compatible not only with physiological buffers but also with high stringency buffers.

Product Details

	RFP-Catcher thus provides great freedom to adjust the binding and washing conditions to the experimental needs.
Bead Ligand:	Antibody
Bead Matrix:	Magnetic Agarose beads
Bead Size:	90 µm

Target Details

Target:	RFP
Alternative Name:	RFP (RFP Products)

Application Details

Application Notes:	Capacity: > 3 µg RFP per µl of packed beads
Protocol:	This protocol provides a general outline of how to use RFP-Catcher (agarose magnetic beads) for immunoprecipitation using a microcentrifuge for sedimentation. Alternatively, it is possible to use RFP-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: RFP-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. 5. Homogenize the RFP-Catcher (agarose magnetic 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 RFP-Catcher (agarose magnetic beads). 8. Place the tubes on a magnet stand until the fluid is clear. Remove the supernatant carefully.

Application Details

9. Repeat wash steps once for a total of two washes.
10. Resuspend equilibrated RFP-Catcher (agarose magnetic beads) gently with the cell lysate supernatant.
11. Rotate the microcentrifuge tubes for 1 h at 4 °C.
12. Place the tubes on a magnet stand until the fluid is clear. Keep a small aliquot of the supernatant as “unbound” fraction. Carefully remove the supernatant.
13. Resuspend RFP-Catcher (agarose magnetic beads) in 1 mL Lysis Buffer.
14. Place the tubes on a magnet stand until the fluid is clear and carefully remove the supernatant.
15. Repeat wash steps twice for a total of three washes.
16. Resuspend RFP-Catcher (agarose magnetic beads) gently in 1 mL TBS.
17. Place the tubes on a magnet stand until the fluid is clear and carefully remove the supernatant.
18. Repeat wash steps once for a total of two washes.
19. Resuspend RFP-Catcher (agarose magnetic beads) resin in 50 µL 2X SDS samples buffer.
20. Heat RFP-Catcher (agarose magnetic beads) resin for 5 min to 95 °C.
21. Place the tubes on a magnet stand until the fluid is clear and transfer the supernatant to fresh microcentrifuge tubes. Keep the RFP-Catcher (agarose magnetic beads) as backup.

Restrictions:	For Research Use only

Handling

Buffer:	50 % slurry in PBS containing 20 % Ethanol
Storage:	4 °C
Storage Comment:	Store at 4 °C, Do not freeze!
Expiry Date:	12 months