Datasheet for ABIN7272855

GFP-Catcher (agarose magnetic beads)

1 Image

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Overview

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

Product Details

Purpose:	GFP-Catcher is based on a high-affinity single-domain antibody (sdAb) that is covalently immobilized on 4% cross-linked magnetic agarose.
Specificity:	GFP (green fuorescent protein) and common GFP derivatives like EGFP, mEGFP, Sirius, tSapphire, Cerulean, eCFP, mTurquoise, acGFP, Emerald, superecliptic pHluorin, paGFP, superfolder GFP, eYFP, mVenus and Citrine. Other not tested.
No Cross-Reactivity:	Does not cross-react with mCherry, dsRed, mRFP, mTagBFP or their most common derivatives.
Characteristics:	GFP-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. GFP-Catcher thus features high affinity and superior capacity for GFP fusion proteins while

Product Details

	showing negligible non-specific background. GFP-Catcher immobilizes a wide range of GFP derivatives. GFP-Catcher is compatible not only with physiological buffers but also with high stringency buffers. GFP-Catcher thus provides great freedom to adjust the binding and washing conditions to the experimental needs.
Material not included:	wash buffers, columns, tubes
Bead Ligand:	Antibody
Bead Matrix:	Magnetic Agarose beads
Bead Size:	90 µm

Target Details

Target:	GFP
Alternative Name:	GFP (GFP Products)

Application Details

Comment:	4% cross-linked magnetic agarose (bead size 50-150 µm) with covalently immobilized single-domain antibody
Protocol:	This protocol provides a general outline of how to use GFP-Catcher (agarose magnetic beads) for immunoprecipitation using a microcentrifuge for sedimentation. Alternatively, it is possible to use GFP-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: GFP-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

Application Details

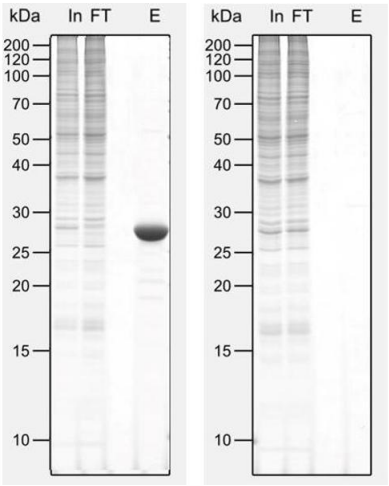
- RNAse A, DNAse I, Benzonase

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 GFP-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 GFP-Catcher (agarose magnetic beads).
8. Place the tubes on a magnet stand until the fluid is clear. Remove the supernatant carefully.
9. Repeat wash steps once for a total of two washes.
10. Resuspend equilibrated GFP-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 GFP-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 GFP-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 GFP-Catcher (agarose magnetic beads) resin in 50 µL 2X SDS samples buffer.
20. Heat GFP-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 GFP-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 for up to 12 months. Do not freeze!
Expiry Date:	12 months



Immunoprecipitation

Image 1. Left: Immunoprecipitation of GFP from E.coli lysate. Right: Immunoprecipitation from E.coli lysate in absence of GFP. In/FT: 1/500 of input and flow through material. E: Eluate from 1 µL of beads.