Datasheet for ABIN5311508

GFP-Catcher

1 Image

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Overview

Quantity:	2000 µL
Target:	GFP
Reactivity:	Aequorea victoria
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 agarose beads.
Sample Type:	Cell Extracts
Specificity:	Recognizes GFP (green fluorescent protein) and common GFP derivatives like EGFP, mEGFP, Sirius, tSapphire, Cerulean, eCFP, mTurquoise, acGFP, Emerald, superecliptic pH luorin, paGFP, superfolder GFP, eYFP, mVenus and Citrine and most common CFP and YFP variants.
Cross-Reactivity (Details):	Does not cross-react with mCherry, mRFP, dsRed, 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 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.
Components:	4 % cross-linked agarose (bead size 50-150 µm) with covalently immobilized single-domain antibody
Material not included:	wash buffers, columns, tubes
Bead Ligand:	Antibody
Bead Matrix:	Agarose beads
Bead Size:	90 µm

Target Details

Target:	GFP
Alternative Name:	GFP (GFP Products)
Target Type:	Viral Protein

Application Details

Protocol:

This protocol provides a general outline of how to use GFP-Catcher (agarose 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.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 $MgCl_2$

Application Details

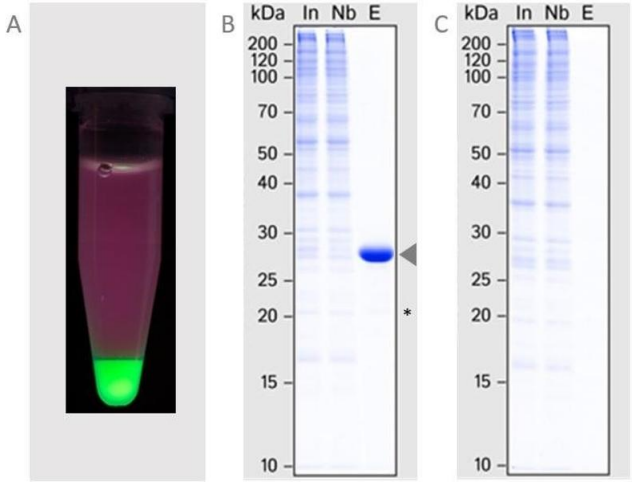
- 100 mM EDTA
- 4 M urea
- 10 mM DTT, 10 mM 2-Mercaptoethanol
- Protease Inhibitors
- RNAse A, DNAse I, Benzonase

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 GFP-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 GFP-Catcher (agarose beads).
8. Centrifuge GFP-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 GFP-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 GFP-Catcher (agarose beads) in 1 mL Lysis Buffer.
14. Centrifuge GFP-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 GFP-Catcher (agarose beads) gently in 1 mL TBS.
17. Centrifuge GFP-Catcher (agarose beads) for 1 min at 1000 x g and carefully remove the supernatant.
18. Resuspend GFP-Catcher (agarose beads) gently in 1 mL TBS.
19. Centrifuge GFP-Catcher (agarose beads) for 1 min at 3000 x g and carefully remove the supernatant.
20. Resuspend GFP-Catcher (agarose beads) resin in 50 µL 2X SDS samples buffer.
21. Heat GFP-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 GFP-Catcher (agarose 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



Immunoprecipitation

Image 1. (A) Pull-down of GFP from a mixture of GFP, mCherry and mTagBFP (B) Immunoprecipitation of GFP (arrow) from HeLa lysate. In/Ft: 1/1000 of input and non-bound material. E: Eluate from 1 µL of beads, *: Specific maturation band from GFP family members (C) Control experiment using functionalized beads lacking sdAbs.

