Datasheet for ABIN5311512

BFP-Catcher

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Overview

Quantity:	2000 µL
Target:	Blue Fluorescent Protein (BFP)
Reactivity:	Entacmaea quadricolor
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:	BFP-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 mTagBFP, mKate, mKate2, mTagRFP, mTagRFP657 and most common fluorescent proteins deriving from Entacmaea quadricolor
Cross-Reactivity (Details):	Does not cross-react with common GFP- or dsRed derivatives.
Characteristics:	BFP-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. BFP-Catcher thus features high affinity and superior capacity for BFP fusion proteins while showing negligible non-specific background. BFP-Catcher is compatible not only with physiological buffers but also with high stringency

Product Details

	buffers. BFP-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:	Blue Fluorescent Protein (BFP)
Alternative Name:	TagBFP (BFP Products)

Application Details

Application Notes:	Coating: sdAb anti-BFP clone 1H7 Matrix: 4 % cross-linked agarose, bead size 50-150 µm Capacity: > 4 µg BFP per µl of packed beads (= 2 µL of slurry) Buffer Compatibility: • Common buffer substances at pH 5 to 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 MgCl2, 100 mM EDTA • 4 M urea • 10 mM DTT, 10 mM 2-Mercaptoethanol • RNAse A, DNAse I, Benzonase, protease inhibitors
Protocol:	This protocol provides a general outline of how to use BFP-Catcher (agarose beads) for immunoprecipitation using a microcentrifuge for sedimentation. Alternatively, it is possible to use BFP-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: BFP-Catcher resins are compatible with commonly used Lysis and Washing

Application Details

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

Restrictions:	For Research Use only

Handling

Buffer:	50 % slurry in PBS containing 20 % Ethanol
Storage:	4 °C

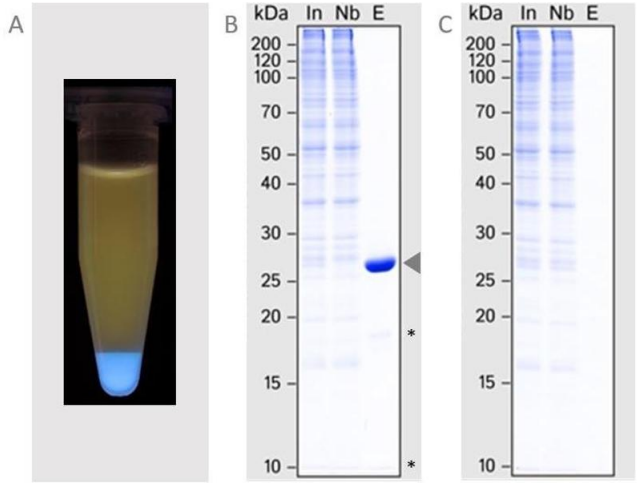
Handling

Storage Comment:	Store at 4 °C, do not freeze
Expiry Date:	12 months

Publications

Product cited in:	Devant, Boršić, Ngwa, Xiao, Chouchani, Thiagarajah, Hafner-Bratkovič, Evavold, Kagan: " Gasdermin D pore-forming activity is redox-sensitive." in: **Cell reports**, Vol. 42, Issue 1, pp. 112008, (2023) (PubMed).

Images



Immunoprecipitation

Image 1. (A) Pull-down of mTagBFP from a mixture of GFP, mCherry and mTagBFP (B) Immunoprecipitation of mTagBFP (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 BFP family members (C) Control experiment using functionalized beads lacking sdAbs.