

Datasheet: TZC041P

Description:	BISPYCATCHER2:HRP
Name:	BISPYCATCHER2
Format:	Catcher-HRP
Product Type:	Recombinant Protein
Quantity:	0.5 mg

Product Details

Applications

This product has been reported to work in the following applications. This information is derived from testing within our laboratories, peer-reviewed publications or personal communications from the originators. Please refer to references indicated for further information. For general protocol recommendations, please visit www.bio-rad-antibodies.com/protocols.

	Yes	No	Not Determined	Suggested Dilution
Immunoassay	▪			

Where this product has not been tested for use in a particular technique this does not necessarily exclude its use in such procedures. Suggested working dilutions are given as a guide only. It is recommended that the user titrates the product for use in their own system using appropriate negative/positive controls.

Target Species	Protein/peptide tag
Product Form	Purified recombinant protein conjugated to Horseradish Peroxidase (HRP) - liquid
Preparation	Recombinant protein expressed in <i>E. coli</i> and purified by affinity chromatography
Source	<i>E. coli</i>
Buffer Solution	Phosphate buffered saline
Preservative Stabilisers	0.0095% MIT
Approx. Protein Concentrations	80 μ M (2.4 mg/ml)

Product Information **BiSpyCatcher2** (H-BiSpyC2) is a 29 kDa homodimer of SpyCatcher2 bridged with a flexible linker. Each SpyCatcher2 subunit can form a stable covalent isopeptide bond with a second protein that includes a SpyTag. The reaction occurs spontaneously on mixing, is rapid, irreversible, high-yielding and shows good specificity. BiSpyCatcher2 contains a His6-tag at the N-terminus. To avoid deamidation of asparagine, a N105D mutation was

introduced ([Hentrich et al. 2020](#)) for each of the two Catchers.

The reaction is robust at pH 5 to 8, at temperatures from +4°C to +37°C, in various buffer conditions (Ca²⁺/Mg²⁺ not needed) and in the presence of detergents. The reaction also occurs inside cells (*in vivo*). Since the speed of the coupling reaction is concentration dependent, it is recommended to use BiSpyCatcher2 undiluted.

All the different formats of BiSpyCatcher2 are compatible with Bio-Rad's recombinant HuCAL® antibodies with a SpyTag2 at the C-terminus of the heavy chain.

[Download BiSpyCatcher coupling protocol](#)

BiSpyCatcher2 is available in two modified versions, BiSpyCatcher2-CYS and BiSpyCatcher2-CYS3, which contain one or three cysteine residues, respectively, for site-specific conjugation by the user. BiSpyCatcher2 is also available conjugated to biotin and HRP.

BiSpyCatcher2-CYS can dimerize by formation of a disulfide bond via the free cysteines. Before conjugation to these cysteines, the BiSpyCatcher2-CYS must be reduced e.g., by addition of 5mM DTT and incubation for 1 hr at room temperature, followed by a fast DTT removal step, e.g., by size exclusion chromatography, as DTT can interfere with the conjugation chemistry.

BiSpyCatcher2-CYS3 contains DTT to avoid oligomerization through disulfide bond formation. Immediately before conjugation, to avoid oxidation and disulfide bond formation, carry out a fast DTT removal step, e.g., by size exclusion chromatography.

[View all available Catchers](#)

Protein Molecular Weight	Predicted molecular weight of the purified Catcher is 29469 Da The molecular weight specified excludes conjugation and will vary upon conjugation depending on the molecular weight of the label
Purity	≥90% determined by SDS-PAGE under reducing conditions and visualized by coomassie blue staining
Instructions For Use	View BiSpyCatcher Coupling Protocol
References	1. Keeble, A.H. <i>et al.</i> (2017) Evolving Accelerated Amidation by SpyTag/SpyCatcher to Analyze Membrane Dynamics. Angew Chem Int Ed Engl. 56 (52): 16521-16525. 2. Hentrich, C. <i>et al.</i> (2021) Periplasmic expression of SpyTagged antibody fragments enables rapid modular antibody assembly. Cell Chem Biol. 28 (6): 813-824.e6.
Storage	Store at -20°C only. Storage in frost-free freezers is not recommended. This product should be stored undiluted. Avoid repeated freezing and thawing as this may denature the protein. Should this product contain a precipitate we recommend microcentrifugation before use.

Guarantee	12 months from date of despatch
Acknowledgements	This product and/or its use is covered by claims of U.S. patents, and/or pending U.S. and non-U.S. patent applications owned by or under license to Bio-Rad Laboratories, Inc. See bio-rad.com/en-us/trademarks for details. His-tag is a registered trademark of EMD Biosciences.
Health And Safety Information	Material Safety Datasheet documentation #20480 available at: https://www.bio-rad-antibodies.com/SDS/TZC041P
Licensed Use	For <i>in vitro</i> research purposes. Any re-sale in any form or any other commercial application needs a written agreement with Bio-Rad.
Regulatory	For research purposes only

Product inquiries: www.bio-rad-antibodies.com/technical-support

To find a batch/lot specific datasheet for this product, please use our online search tool at: bio-rad-antibodies.com/datasheets
'M450521:260325'

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