

Datasheet: TZC002

Description:	BiSpyCatcher2
Name:	BISPYCATCHER2
Format:	Catcher
Product Type:	Recombinant Protein
Quantity:	1 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 <a href="www.bio-rad-antibodies.com/protocols">www.bio-rad-antibodies.com/protocols</a>.

	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	BiSpyCatcher2 purified recombinant protein - liquid	
Preparation	Recombinant protein expressed in E. coli and purified by affin	nity chromatography
Source	E.coli	
Buffer Solution	Phosphate buffered saline	
Preservative Stabilisers	0.1% ProClin 950	
Approx. Protein Concentrations	80 μM (2.3 mg/ml)	

#### **Product Information**

**BiSpyCatcher2** (H-BiSpyC2) is a 29 kDa homodimer of SpyCatcher2 (<u>TZC001</u>) 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 (<u>TZC002</u>) is available in two modified versions, BiSpyCatcher2-CYS (<u>TZC002CYS</u>) and BiSpyCatcher-CYS3 (<u>TZC002CYS3</u>), which contain one or three cysteine residues, respectively, for site-specific conjugation by the user. BiSpyCatcher2 (<u>TZC002</u>) is also available conjugated to biotin (<u>TZC002B</u>) and HRP (<u>TZC002P</u>).

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 29018 Da
Purity	≥90% determined by SDS-PAGE under reducing conditions and visualized by coomassie blue staining
Instructions For Use	View BiSpyCatcher Coupling Protocol
References	<ol> <li>Keeble, A.H. <i>et al.</i> (2017) Evolving Accelerated Amidation by SpyTag/SpyCatcher to Analyze Membrane Dynamics. <u>Angew Chem Int Ed Engl. 56 (52): 16521-16525.</u></li> <li>Hentrich, C. <i>et al.</i> (2021) Periplasmic expression of SpyTagged antibody fragments enables rapid modular antibody assembly. <u>Cell Chem Biol. 28 (6): 813-824.e6.</u></li> </ol>
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	ProClin is a trademark of The Dow Chemical Company ("Dow") or an affiliated company of Dow 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.
Health And Safety Information	Material Safety Datasheet documentation #20480 available at: <a href="https://www.bio-rad-antibodies.com/SDS/TZC002">https://www.bio-rad-antibodies.com/SDS/TZC002</a> 20480
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

## **Related Products**

### **Recommended Useful Reagents**

BiSpyCatcher2:Biotin (TZC002B)

BiSpyCatcher2-CYS (TZC002CYS)

BiSpyCatcher2-CYS3 (TZC002CYS3)

BiSpyCatcher2:HRP (TZC002P)

SpyTag3 PEPTIDE (BLP086)

ANTI SpyCatcher (HCA379)

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To find a batch/lot specific datasheet for this product, please use our online search tool at: bio-rad-antibodies.com/datasheets 'M422627:230921'

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