

Datasheet: TZC041P

**BATCH NUMBER 64697328**

<b>Description:</b>	BISPYCATCHER2:HRP
<b>Name:</b>	BISPYCATCHER2
<b>Format:</b>	Catcher-HRP
<b>Product Type:</b>	Recombinant Protein
<b>Quantity:</b>	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](http://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.

<b>Target Species</b>	Protein/peptide tag
<b>Product Form</b>	Purified recombinant protein conjugated to Horseradish Peroxidase (HRP) - liquid
<b>Preparation</b>	Recombinant protein expressed in <i>E. coli</i> and purified by affinity chromatography
<b>Source</b>	<i>E. coli</i>
<b>Buffer Solution</b>	Phosphate buffered saline
<b>Preservative Stabilisers</b>	0.0095% MIT
<b>Approx. Protein Concentrations</b>	80 $\mu$ 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 (Ca<sup>2+</sup>/Mg<sup>2+</sup> 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](#)

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<b>Protein Molecular Weight</b>	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
<b>Purity</b>	≥90% determined by SDS-PAGE under reducing conditions and visualized by coomassie blue staining
<b>Instructions For Use</b>	<a href="#">View BiSpyCatcher Coupling Protocol</a>
<b>References</b>	1. Keeble, A.H. <i>et al.</i> (2017) Evolving Accelerated Amidation by SpyTag/SpyCatcher to Analyze Membrane Dynamics. <a href="#">Angew Chem Int Ed Engl. 56 (52): 16521-16525.</a> 2. Hentrich, C. <i>et al.</i> (2021) Periplasmic expression of SpyTagged antibody fragments enables rapid modular antibody assembly. <a href="#">Cell Chem Biol. 28 (6): 813-824.e6.</a>
<b>Storage</b>	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.

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**Guarantee** 12 months from date of despatch

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His-tag is a registered trademark of EMD Biosciences.

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**Health And Safety Information** Material Safety Datasheet documentation #20480 available at: <https://www.bio-rad-antibodies.com/SDS/TZC041P>

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**Regulatory** For research purposes only

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**Product inquiries:** [www.bio-rad-antibodies.com/technical-support](http://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](http://bio-rad-antibodies.com/datasheets)  
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