

Datasheet: TZC001B BATCH NUMBER 167169

| Description: | SpyCatcher2:Biotin |
|---------------|---------------------|
| Name: | SPYCATCHER2 |
| Format: | Catcher-Biotin |
| 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 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 Biotin - liquid | |
| Preparation | Recombinant protein expressed in E. coli and purified by affin | ity chromatography |
| Source | E.coli | |
| Buffer Solution | Phosphate buffered saline | |
| Preservative Stabilisers | 0.0095% MIT | |
| Approx. Protein Concentrations | 250 μM (3.9 mg/ml) | |

Product Information

SpyCatcher2 (H-SpyC2) is a 15.7 kDa protein that forms 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. SpyCatcher2 contains a His6-tag at the N-terminus. To avoid deamidation of asparagine, a N105D

mutation was introduced (Hentrich et al. 2020).

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 SpyCatcher2 undiluted.

All the different formats of SpyCatcher2 are compatible with Bio-Rad's recombinant HuCAL® antibodies with a SpyTag2 at the C-terminus of the heavy chain.

Download SpyCatcher coupling protocol

SpyCatcher2 (TZC001) is available conjugated to biotin (TZC001B). It is also available as a modified version, SpyCatcher2-CYS (TZC001CYS), which contains a cysteine residue for site-specific conjugation by the user.

SpyCatcher2-CYS can dimerize during storage by formation of a disulfide bond via the free cysteines. Before conjugation to these cysteines, the SpyCatcher2-CYS dimers should be mildly 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 using PD-10 desalting columns, as DTT can interfere with the subsequent conjugation chemistry. Dialysis is not recommended for DTT removal, as the cysteines can oxidize again during this slow process.

View all available Catchers

| Protein Molecular Weight | Predicted molecular weight of the purified Catcher is 15904 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 SpyCatcher Coupling Protocol |
| References | 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): 16521-5.</u> 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> |
| 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/TZC001B 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

SpyCatcher2 (TZC001)
SpyCatcher2-CYS (TZC001CYS)
ANTI SpyCatcher (HCA379)
SpyTag3 PEPTIDE (BLP086)

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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 'M422625:230921'

Printed on 17 Apr 2024

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