

Datasheet: TZC025CYS

BATCH NUMBER 160923

Description:	SpyCatcher3-CYS
Name:	SPYCATCHER3
Format:	Catcher-CYS
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	SpyCatcher3 purified recombinant protein, with an engineered cysteine residue; used for site-specific chemical conjugation to a label of choice - liquid
Preparation	Recombinant protein expressed in <i>E. coli</i> and purified by affinity chromatography
Buffer Solution	Phosphate buffered saline
Preservative	0.1% ProClin 950
Stabilisers	5 mM EDTA
Approx. Protein Concentrations	455 µM (7 mg/ml)

Product Information **SpyCatcher3** (H-SpyC3) is a 15.2 kDa protein that forms a stable covalent isopeptide bond with a second protein that includes a SpyTag ([Keeble et al. 2019](#)). The reaction occurs spontaneously on mixing, is rapid, irreversible, high-yielding and shows good specificity. SpyCatcher3 contains a His6-tag at the N-terminus. 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 SpyCatcher3 undiluted.

All the different formats of SpyCatcher3 are compatible with Bio-Rad's recombinant HuCAL® antibodies with a SpyTag2 at the C-terminus of the heavy chain.

SpyCatcher3 couples faster to SpyTag than the earlier SpyCatcher versions. At neat concentrations the difference in speed between SpyCatcher3 and SpyCatcher2 is negligible but with diluted SpyTag and/or SpyCatcher using SpyCatcher3 shows some benefits. Therefore, Spycatcher3 is recommended for immobilization on plates or beads to bind a capture antibody or other proteins with a SpyTag.

[Download SpyCatcher coupling protocol](#)

SpyCatcher3 (TZC025) is available as a modified version, SpyCatcher3-CYS (TZC025CYS), which contains a cysteine residue for site-specific conjugation by the user

SpyCatcher3-CYS can dimerize by formation of a disulfide bond via the free cysteines. Before conjugation to these cysteines, the SpyCatcher3-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. 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 15368 Da
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Purity	≥90% determined by SDS-PAGE under reducing conditions and visualized by coomassie blue staining
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Instructions For Use	View SpyCatcher Coupling Protocol
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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.
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Guarantee	12 months from date of despatch
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Health And Safety Information Material Safety Datasheet documentation #20484 available at:
<https://www.bio-rad-antibodies.com/SDS/TZC025CYS>
20484

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Recommended Useful Reagents

[SpyCatcher3 \(TZC025\)](#)

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