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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The SiMPLe CHC Antibody Labeling Kit leverages Sortase Mediated Protein Ligation to specifically label recombinant antibodies containing the sequence LPXTG at the C-terminus of the heavy chain (CHC). A diverse number of poly-Glycine $(G)_n$ molecules and labels (e.g. fluorophores, biotin, enzymes, peptides, etc.) are compatible with this kit. The site-specific conjugation ensures that the antigen binding site remains available for target binding and reduces heterogeneity compared to chemical conjugation methods, making it ideal for Antibody-Drug Conjugate (ADC) development and flow cytometry. The kit provides reagents to label 3 x 100 µg of recombinant antibody, as well as purification columns to aid in the removal of excess poly-Glycine $(G)_n$-label. Anti-HER2-LPETGH_6 and GGGK-FITC are included as positive controls.

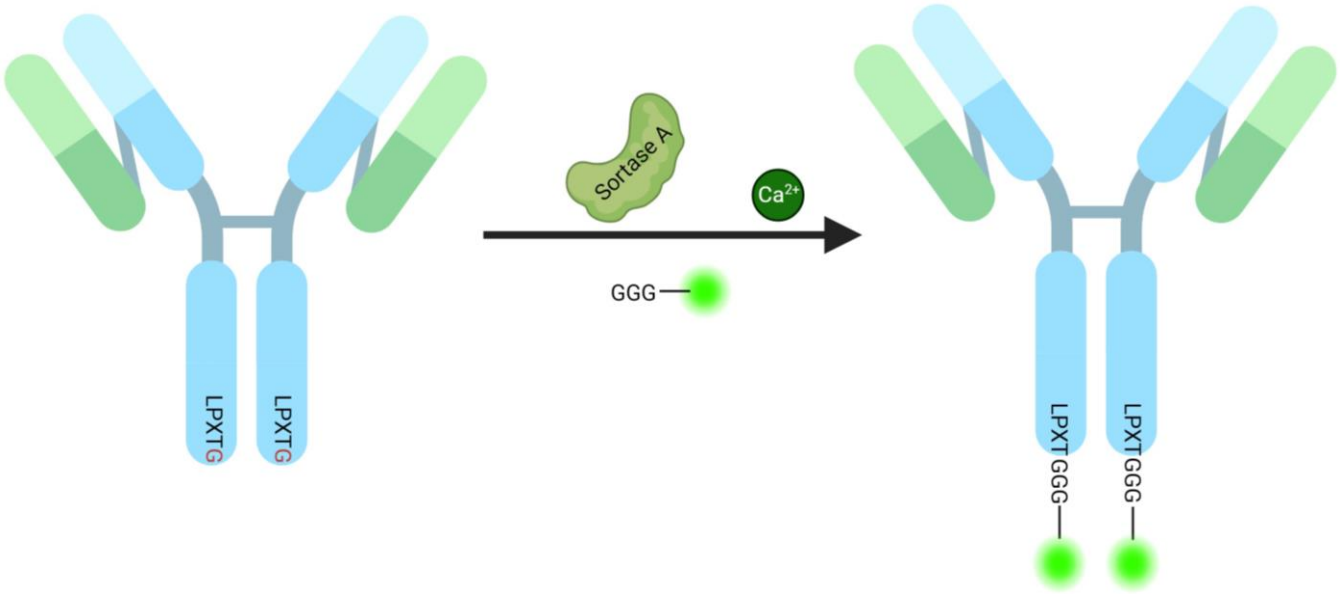

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Figure 1: Illustration of the mechanism of targeted labeling using the SiMPLe CHC Antibody Labeling Kit (Sortase Mediated Protein Ligation).

Each antibody contains two Sortase recognition sequences, so a maximum of two labels per antibody can be achieved.

Background

Staphylococcal Sortase A is a bacterial transpeptidase that covalently attaches proteins to the bacterial cell wall, maintaining bacterial virulence and infectivity. Sortase A cleaves a specific peptide sequence (LPXTG recognition motif) within a target protein between threonine and glycine, with a strong preference for terminal locations in proteins. The cysteine residue of the active site forms a transient thioacyl intermediate complex with the substrate protein. This intermediate complex is then immediately attacked by oligo-glycine nucleophiles present on peptide-glycans of the bacterial wall to form an amide bond. This transpeptidase activity can be used for protein labeling. Using a recombinantly expressed antibody containing a C-terminal Sortase recognition sequence (LPXTG for S. *aureus* Sortase A) and a highly active Sortase A Heptamutant, the direct conjugation of poly-Glycine $(G)_n$-labels to the target protein can be achieved.

Applications
Site-specific labeling of recombinant antibodies.

Supplied Materials

Catalog #	Name	Amount	Storage
71048	Sortase A Heptamutant, His-Tag	3 Vials (1U/Vial)	-80°C
79394	Reaction Buffer	100 µl	4°C
82197	Stop Solution	20 µl	4°C
79396	Purification Columns	3	Room Temp
79397	Collection Tubes	6	Room Temp
101689	Anti-HER2-LPETGH$_6$	1 Vial	-80°C
82198	GGG-FITC	1 Vial	-80°C

Materials Required but Not Supplied

- Antibody of interest containing the sequence LPXTG in the C-terminus of the heavy chain (1 mg/ml, 100 µg per reaction)
- Poly-Glycine-Label (125 µM per reaction, 3.75 mM stock)
- Microcentrifuge
- Buffer for final formulation (such as PBS or TBS)

Storage Conditions

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Reaction Protocol

- The conditions described below are a general guideline. Further optimization of the amounts to be used may be required depending on the label and antibody.
- It is recommended to prepare the purification column 15-20 minutes prior to use (Step 2).
- Although the addition of the Stop Solution will inhibit Sortase activity, we recommend Sortase removal from the conjugated antibody for long-term storage.

Step 1: Reaction Setup

1. Thaw one vial of **Sortase A Heptamutant** per reaction on ice.

   Note: Maintain on ice while setting up the reaction.

2. Add the following reagents to the vial of Sortase designated for the antibody and label of interest:

   2.1. Add 15 µl of Reaction Buffer.

   2.2. Add 5 µl of 3.75 mM Poly-Glycine Label.

   2.3. Add 100 µl of the antibody of interest (1 mg/ml).

3. Add the following reagents to the vial of GGG-FITC designated for the control (optional):

   3.1. Add 15 µl of Reaction Buffer.

   3.2. Add vial of Sortase.

   3.3. Add vial of Anti-HER2-LPETGH_6.

4. Mix by gently pipetting up and down.

5. Incubate the reaction at Room Temperature (RT) for 1 hour and mix every 5-10 minutes.

Step 2: Column Setup

1. Prepare one column for each reaction.

2. Remove the bottom plug and loosen cap (do not remove cap).

3. Place column in a clean collection tube and centrifuge at 1,500 *x g* for 1 minute to remove storage buffer.

4. Discard flow through and put the column back in the collection tube.

5. Wash/equilibrate the purification column by adding 300 µl of desired buffer on top of the resin (take care not to disturb resin).

6. Centrifuge at 1,500 *x g* for 1 minute and discard flow through as before.

7. Repeat the wash step two additional times, using a 2-minute spin for the final wash.

8. Blot the bottom of the column to remove excess buffer and transfer column to a clean collection tube.

Step 3: Excess Label Removal

1. Apply the entire reaction to the top of the column resin.

2. Centrifuge column at 1,500 *x g* for 2 minutes and retain the flow-through, as it contains the purified sample.

3. Add 5 µl of Stop Solution to the flow through and mix well.

4. Use the labeled antibody immediately, or add glycerol to 20% and store for up to 6 months at -80°C.

Calculations

The calculations described apply to the control antibody and label provided. Modifications may be required for other antibodies and labels.

Degree of Labeling (DOL) can be determined as follows:

$$DOL = (2.77 \times A_{495}) / [A_{280} - (0.35 \times A_{495})]$$

The concentration of the conjugate antibody-FITC can be calculated as follows:

$$IgG\ (mg/ml) = [A_{280} - (0.35 \times A_{495})] / 1.4$$

Where 1.4 is the A_{280} of most IgG at a concentration of 1 mg/ml at pH 7.0.

**We anticipate >50% yield with a DOL greater than 1, but less than 2, as steric hindrance and other factors may prohibit the labeling of both sites. Results are substrate- and label- dependent and may vary.*

Validation Data

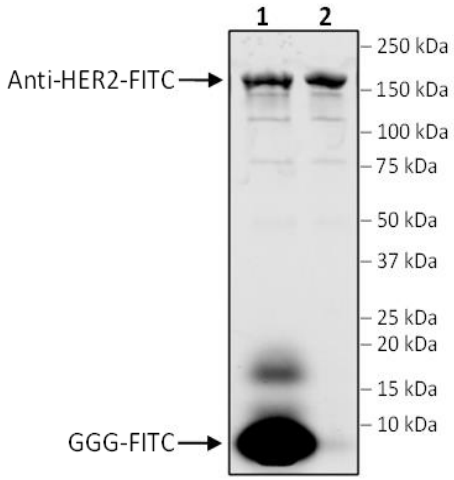

Figure 2: SDS-PAGE fluorescence-based analysis of anti-HER2 labeling with GGG-FITC.
Anti-HER2-FITC and free GGG-FITC are both detected prior to spin column purification (Lane 1). After spin column purification, >99% of the free GGG-FITC is removed (Lane 2).

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

References

Popp M., 2015 *Methods Mol Biol*. 1266:185-98.

Related Products

Products	*Catalog #*	*Size*
Sortase A Pentamutant, His-Tag Recombinant	71046	50 µg
Sortase A Hexamutant, His-Tag Recombinant	71047	50 µg
Sortase A Octamutant, His-Tag Recombinant	72518	50 µg
Sortase A, *S. aureus*, His-Tag Recombinant	71086	50 µg
Sortase Sampling Kit	79709	50 µg
Ca^{2+} Independent Sortase, His-Tag (*S. aureus*) Recombinant	100666	100 µg/1 mg

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