Description

The SiMPLe Protein Labeling Kit (Sortase Mediated Protein Ligation) is a kit designed to label recombinant antibodies or proteins that contain the sortase recognition sequence, making use of the highly active Sortase A Pentamutant and resulting in a labeling efficiency of greater than 90%. This kit provides enough reagents, including purification columns to remove Sortase A Pentamutant and excess label, to label 3 x 100 µg of recombinant antibody/protein. Ubiquitin-LPETGH$_6$ and GGG-Clover are included as positive controls.

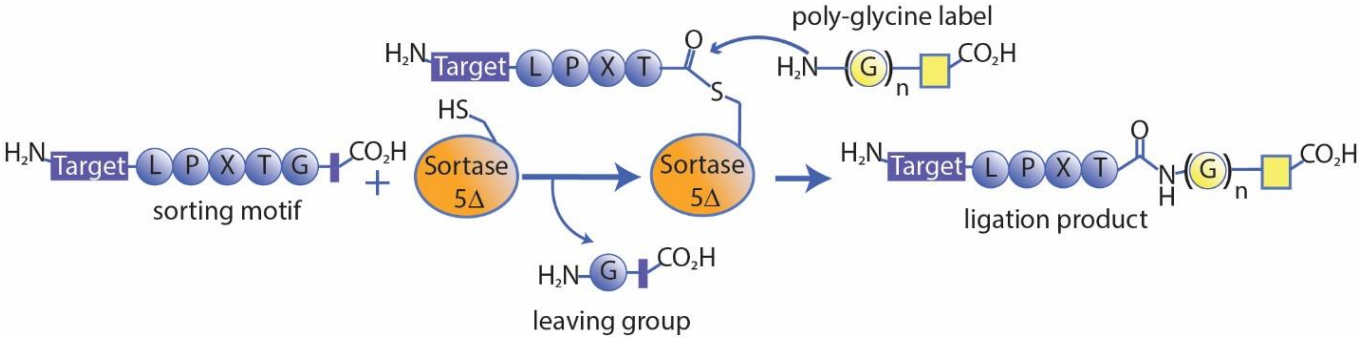

Figure 1: Schematic of the mechanism of action used in the SiMPLe Protein Labeling Kit (Sortase Mediated Protein Ligation).

The combination of your protein of interest with Sortase A Pentamutant and a poly-glycine label results in a labeled protein, when incubated for 30 minutes at 37°C. This labeled protein can then be purified.

Background

Sortases catalize a transpeptidation reaction, where the enzyme cleaves the amide bond between the threonine and glycine of the sortase recognition sequence (LPXTG for S. *aureus* Sortase A), generating a thioacyl intermediate. Subsequently, this intermediate is resolved by the N-terminus of an oligoglycine nucleophile, creating a new peptide bond that links the substrate to the incoming nucleophile.

Applications

Site-specific labeling of recombinant antibodies and proteins.

Supplied Materials

Catalog #	Name	Amount	Storage
71046	Sortase A Pentamutant, *S. aureus*, His-Tag*	3 vials (1U/Vial)	-80°C
79394	Reaction Buffer	100 µl	4°C
79395	Stop Solution	200 µl	4°C
79396	Purification Columns	3	Room Temperature
79397	Collection Tubes	6	Room Temperature
79398	Ubiquitin-LPETGH$_6$	1 Vial	-80°C
79399	GGG-Clover	1 Vial	-80°C

**The concentration of the protein is lot-specific and will be indicated on the tube.*

BPS Bioscience
● bpsbioscience.com ● 858-202-1401 ● support@bpsbioscience.com

Materials Required but Not Supplied

- Target Protein (1 mg/ml, 100 µg per reaction)
- Poly-glycine label (5 mM, 5 µl per reaction, <10 kDa)
- Microcentrifuge
- Buffer for final formulation (such as PBS or TBS)
- 37°C incubator

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.

Assay Protocol

1. Thaw a single vial of **Sortase A Pentamutant** on ice.

   Note: Maintain on ice while setting up the reaction.

2. Add 100 µl of target protein at 1 mg/ml to the vial containing Sortase A Pentamutant).

3. Add 20 µl of Reaction Buffer.

4. Add 5 µl of Poly0Glycine Label (5 mM).

5. Mix by gently pipetting up and down.

6. Incubate reaction at 37°C for 30 minutes and mix every 5-10 minutes (or place on shaker if available).

7. Resuspend the Stop Solution.

8. Add 50 µl to the reaction.

9. Incubate on ice for 30 minutes (mixing every 5-10 minutes).

10. Set up the purification column to remove to remove Sortase A Pentamutant and excess label:

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

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

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

10.4 Equilibrate the purification column by adding 300 µl of desired buffer on top of the resin (take care not to disturb resin).

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

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

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

11. Mix the reaction by gently pipetting and then apply the entire reaction to the top of the column resin.

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

13. Use the sample immediately, or add glycerol to 20% and store up to 6 months at -80°C.

Component	**Volume**
Target Protein (1 mg/ml)	100 µl
Reaction Buffer	20 µl
Poly-Glycine Label (5 mM)	5 µl
Sortase A Pentamutant	1 vial
Total	**200 µl**

Example Results

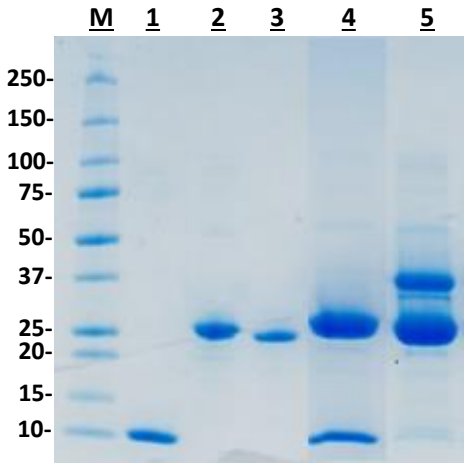

Figure 2: Analysis by SDS-PAGE of Ubiquitin-LPETGH$_6$ and GGG-Clover labeling using the SiMPLe Protein Labeling Kit (Sortase Mediated Protein Ligation).
Ubiquitin-LPETGH$_6$ (Lane 1, 10 kDa), GGG-Clover (Lane 2, 27 kDa) and Sortase A Pentamutant (Lane 3, 22 kDa) very run side by side with Ubiquitin-LPETGGG-Clover product at t= 0 (lane 4) and after 1 hour (Lane 5, 37 kDa). After combining the reagents and incubating for 1 hour at 37°C, labelling efficiency is >90%, as demonstrated by the disapperance of free Ubiquitin-LPETGH$_6$ remaining after 1 hour (Lane 5) compared to time = 0 (Lane 4). The excess GGG-Clover is retained after the column purification step, as its molecular weight is greater than the molecular weight cut-off of the purification column (10 kDa).

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 email support@bpsbioscience.com

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

Related Products

Products	*Catalog #*	*Size*
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