



# SZABO SCANDIC

Part of Europa Biosite

## Produktinformation



Forschungsprodukte & Biochemikalien



Zellkultur & Verbrauchsmaterial



Diagnostik & molekulare Diagnostik



Laborgeräte & Service

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See the following pages for more information!



### Lieferung & Zahlungsart

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### Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

### SZABO-SCANDIC HandelsgmbH

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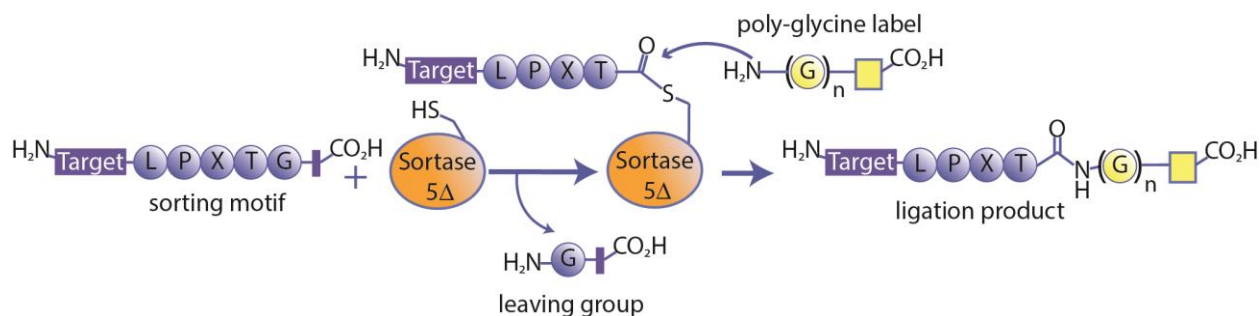
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**Data Sheet**  
**SiMPLe Protein Labeling Kit**  
 Sortase Mediated Protein Ligation  
 Catalog # 79392

**DESCRIPTION:**

Sortases catalyze 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. The Sortagging Kit takes advantage of this reaction to label virtually any recombinant antibody or protein containing the sortase recognition sequence. Labeling efficiency of greater than 90% is achieved by taking advantage of the highly active Sortase 5Δ Pentamutant. Simply combine your protein of interest with Sortase 5Δ and a poly-glycine label, and incubate for 30 minutes at 37°C. Purification columns are included to remove Sortase 5Δ and excess label. The kit also includes Ubiquitin-LPETGH<sub>6</sub> and GGG-Clover as positive controls.



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Each kit provides sufficient materials to label 3x 100 µg recombinant antibody/protein.

**COMPONENTS:**

Catalog #	Component	Amount	Storage
79393	Sortase 5Δ enzyme	3 Vials (1U/vial)	-80°C (Avoid freeze/thaw cycles)
79394	Reaction Buffer	100 µl	4°C
79395	Stop Solution	200 µl	4°C
79396	Purification Columns	3	Room Temp
79397	Collection Tubes	6	Room Temp
79398	Ubiquitin-LPETGH <sub>6</sub>	1 vial	-80°C
79399	GGG-Clover	1 vial	-80°C

**MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:**

Target protein (1 mg/mL, 100 µg per reaction)  
Poly-glycine label (5 mM, 5 µl per reaction, <10 kDa)  
37°C incubator  
Microcentrifuge  
Final formulation buffer (e.g. PBS or TBS)

**APPLICATIONS:** Great for the site-specific labeling of recombinant antibodies and proteins.

**STABILITY:** At least 6 months from date of receipt, when stored as directed.

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

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## LABELING PROTOCOL:

- 1) Thaw a single vial of Sortase 5 $\Delta$  on ice.
- 2) While on ice, set up the labeling reaction as follows (add all reagents to the vial containing Sortase 5 $\Delta$ ):

Reagent	Volume to add
Target Protein (1 mg/mL)	100 $\mu$ l
Reaction Buffer	20 $\mu$ l
Poly-Glycine Label (5 mM)	5 $\mu$ l
Sortase 5 $\Delta$	1 vial
<b>Total</b>	<b>200 <math>\mu</math>l</b>

- 3) Mix reaction well by gently pipetting up and down.
- 4) Incubate reaction at 37°C for 30 minutes and mix every 5-10 minutes (or place on shaker if available).
- 5) Resuspend the stop solution, transfer 50  $\mu$ L to the reaction and incubate on ice for 30 minutes (mixing every 5-10 minutes).
- 6) Prepare one purification column to remove Sortase 5 $\Delta$  and excess label:
  - Remove bottom plug and loosen cap (do not remove cap).
  - Place column in a clean collection tube and centrifuge at 1,500 x g for 1 minute to remove storage buffer.
  - Discard flow through and replace the column back in the collection tube.
  - Equilibrate the purification column by adding 300  $\mu$ l of desired buffer on top of the resin (take care not to disturb resin). Centrifuge at 1,500 x g for 1 minute and discard flow through as before. Repeat this step 2 additional times, using a 2 minute spin for the final wash.
  - Blot the bottom of the column to remove excess buffer and transfer column to a clean collection tube.
- 7) Mix the reaction by gently pipetting and then apply the entire reaction on top of the column resin.

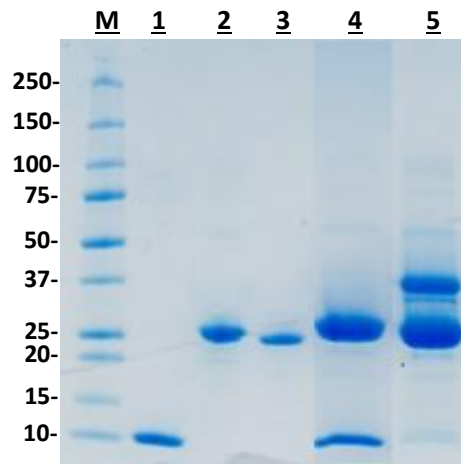
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- 8) Centrifuge column at 1,500 x g for 2 min and retain the flow-through, as it contains the purified sample.
- 9) Use the sample immediately, or add glycerol to 20% and store up to 6 months at -80°C.

**EXAMPLE OF LABELING RESULTS:**

This kit was optimized using Ubiquitin-LPETGH<sub>6</sub> (Lane 1, 10 kDa), GGG-Clover (Lane 2, 27 kDa) and Sortase 5 $\Delta$  (Lane 3, 22 kDa). After combining the reagents and incubating for 1 hour at 37°C, the Ubiquitin-LPETGGG-Clover product is observed (Lane 5, 37 kDa). Labelling efficiency is >90%, as demonstrated by the disappearance of free Ubiquitin-LPETGH<sub>6</sub> remaining after 1 hour (Lane 5) compared to time = 0 (Lane 4). Note: 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).

**TROUBLESHOOTING:**

In the event labeling of your protein of interest is not observed, we have included Ubiquitin-LPETGH<sub>6</sub> and GGG-Clover as positive controls. Simply use these in place of your reagents to optimize reaction conditions. *For questions or concerns, please contact BPS Bioscience, Inc. at [info@bpsbioscience.com](mailto:info@bpsbioscience.com)*

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**RELATED PRODUCTS:**

<b><u>Product</u></b>	<b><u>Cat. #</u></b>	<b><u>Size</u></b>
Sortase A Pentamutant, S. aureus, His-tag	71046	50 µg
Sortase A Hexamutant, His-tag	71047	50 µg
Sortase A Heptamutant, His-tag	71048	50 µg
Sortase A Octamutant, His-tag	72518	50 µg
Sortase A, S. aureus, His-tag	71086	50 µg

BPS is a leader in the custom expression of recombinant proteins. Contact us about custom projects for the expression of target proteins containing the sortase recognition sequence.

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