Sortag-IT™ TEV-Biotin Labeling Kit

Catalog No.: 13106
Format: 3 x 100 µg

Description

The Sortag-IT™ Biotin Labeling Kits* are designed to label Active Motif's highly specific AbFlex™ recombinant antibodies (rAb) via the Sortase tag recognition sequence (LPXTG) that is incorporated into the heavy chains of each AbFlex antibody. Sortase A belongs to the sortase family of transpeptidases found in Gram-positive bacteria and is used to catalyze the attachment of poly-Glycine containing labels to the recognition sequence. The Sortag-IT Labeling Kit uses Active Motif's Sortase A5 pentamutant sortase which has activity >15 times wild-type Sortase, allowing for a faster, more efficient labeling reaction. Each antibody contains two Sortase tag sequences and can add a maximum of two labels per antibody. Simply combine your AbFlex antibody with the poly-Glycine label, add Sortase A5, and incubate for 1 hour at 30°C. Purification columns are included to remove excess label and Stop Solution is provided to inactivate the Sortase A5 enzyme. Labeled antibodies are ready for downstream analysis or can be stored at 4°C for up to 3 months.

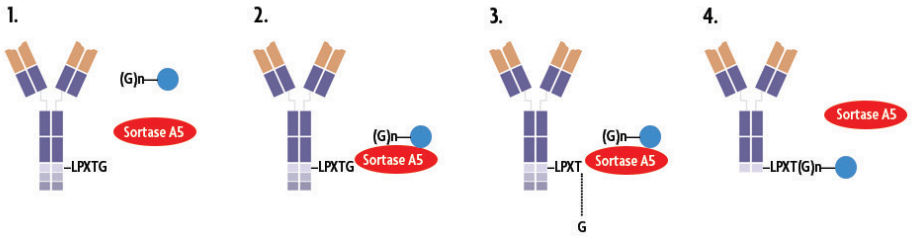


Figure 1: The Sortag-IT™ antibody labeling system.

(1) Combine the AbFlex™ recombinant antibody (rAb) of interest with the desired poly-Glycine $(G)_n$ label at a ratio of greater than or equal to 5 nmol label to 1 nmol antibody in the presence of Sortase A5 enzyme and reaction buffer. (2) Sortase A5 will bring poly-Glycine label to the Sortase recognition sequence (LPXTG) on the antibody. (3) Sortase A5 will cleave the bond between the Threonine and the Glycine of the LPXTG recognition sequence creating an acyl enzyme intermediate which allows for the attachment of the poly-Glycine label. (4) Following the reaction, the labeled AbFlex antibody can be purified away from the free label and the Sortase A5 enzyme inactivated with Stop Solution.

The TEV-Biotin label is designed to provide a cleavable release of the AbFlex antibody from biotin using the Tobacco etch Virus (TEV) protease. This labeling strategy enables antibody capture using biotin/streptavidin followed by release of the antibody via TEV cleavage.

Note: When performing a TEV cleaving following antibody labeling, we suggest to omit the use of DTT in the TEV cleavage reaction and substitute it with 3 mM glutathione. The use of DTT in the TEV reaction can reduce the disulfide bond in the antibody heavy and light chains, while glutathione will not interfere with the antibody structure.

Contents

Each Sortag-IT™ TEV-Biotin Labeling Kit provides sufficient materials to label 3 x 100 µg AbFlex™ recombinant antibody with a yield > 50%.

- 3 units Sortase A5 enzyme; Store at -80°C
- 1 ml Reaction Buffer AM3; Store at 4°C
- 5 mM $(Gly)_5$- TEV-Biotin label; Store at -20°C
- 10 µl Stop Solution AM3; Store at RT
- 3 ea Purification columns; Store at RT

Items Required but not included

- 100 µg AbFlex™ Recombinant antibody per reaction (see www.activemotif.com/abflex for a complete product listing)
- 1.5 ml microcentrifuge tubes and microcentrifuge
- Thermomixer, or equivalent instrument capable of incubating samples at 30°C with shaking
- PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4

*** US Patent 9,267,127**

Procedure for Sortag-IT TEV-Biotin:
Reaction conditions are label-specific.

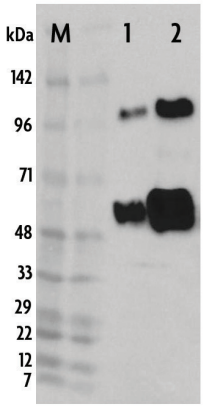
1. Thaw the vial of Sortase A5 enzyme on ice.
2. Set up the labeling reactions in a 1.5 ml microcentrifuge tube. Add reagents in the order shown below and mix by pipetting.

Reagent	Volume to add
AbFlex antibody (1 µg/µl)	100 µl
Reaction Buffer AM3	94 µl
$(Gly)_5$-TEV-Biotin label (5 mM)	5 µl
Sortase A5 enzyme (1 unit/µl)	1 µl
Total Volume	**200 µl**

3. Incubate the labeling reactions at 30°C for 1 hour with shaking at 1000 rpm.
4. Following the incubation, remove excess label from the reaction by purification using the included columns.
   a. Equilibrate each column by adding 450 µl PBS.
   b. Spin columns at 13,500 x g for 5 minutes in a microcentrifuge. Discard flow through and replace column in the collection tube.
   c. Add 250 µl PBS to each antibody labeling reaction (450 µl final volume).
   d. Add diluted labeling reactions to the equilibrated column.
   e. Spin columns at 13,500 x g for 5 minutes in a microcentrifuge. Discard flow through and replace column in the collection tube.
   f. Add 450 µl PBS to wash the column. Spin columns at 13,500 x g for 5 minutes in a microcentrifuge. Discard flow through and replace column in the collection tube.
   g. Repeat the wash from step f two additional times for a total of three washes.
   h. Add 100 µl PBS to the column. Place the column inverted into a new microcentrifuge tube.
   i. Spin columns at 800 x g for 2 minutes to collect labeled antibody.
5. Add 2.5 µl Stop Solution AM3 to each labeled antibody to inactivate the Sortase A5 enzyme.
6. Use immediately, or store at 4°C for up to 3 months.

Appendix

Application Data



Western blot of Histone H3K9ac-TEV-Biotin directly conjugated antibody.

The Sortag-IT™ TEV-Biotin Labeling Kit was used to directly conjugate 100 µg of Active Motif's AbFlex™ Histone H3K9ac recombinant antibody with Biotin or TEV-Biotin. One µg Histone H3K9ac-TEV-Biotin (Lane 1) and 1 µg Histone H3K9ac-Biotin (Lane 2) were loaded onto a 4-12% SDS-PAGE gel and run at 150 V for 1.5 hours. Material was transferred to a nitrocellulose membrane and blocked with TBST containing 5% milk. The blot was then probed with 3 µl Streptavidin-HRP (BioLegend, Cat 405210) for 1 hour at room temperature. Blots were washed three times with TBST and developed using the Bio-Rad Clarity kit. Results show the detection of the biotin labeled antibody by Streptavidin-HRP. The heavy chain of the antibody is shown at ~ 50 kDa, while the upper band at ~100 kDa represents antibody that did not disassociate (heavy and light chains combined).

Troubleshooting

Problem/question	Recommendation
How can I cleave the TEV-biotin linker?	The Tobacco Etch Virus (TEV) protease can be used to cleave the TEV-Biotin linker and release the antibody from the biotin label. Follow the recommended reaction conditions of the TEV protease supplier to perform the cleavage.
How can I determine the labeling efficiency?	The maximum number of labels that can be added to an AbFlex antibody is 2 (this is a theoretical maximum as steric hindrance may prohibit the addition of 2 labels per antibody molecule depending on the label used). Commercially available biotin quantification kits are designed to detect antibodies that have been chemically labeled and, therefore, contain a higher number of biotin molecules per antibody. Attempting to detect only 2 biotins per antibody will fall below the limit of detection and we do not suggest using these biotin quantification kits. Instead, we suggest a functional assay to detect the presence of the biotin labeled antibody. (*e.g.* Western blot with Streptavidin detection)
Can I label for shorter or longer time periods?	The Sortag-IT Labeling Kits have been optimized for the most efficient labeling time based on the use of 100 µg of antibody with the recommended amount of poly-Glycine label per reaction. Altering the labeling time may result in decreased labeling efficiency.
Can I label smaller amounts of antibody?	Yes, smaller amounts of antibody (25 -100 µg) can be labeled with the Sortag-IT Labeling Kits, but the amount of Sortase A5 enzyme and poly-Glycine label should not be modified in the reaction. Volume differences should be corrected using Reaction Buffer AM3 to maintain a total volume of 200 µl per reaction. Optimization of labeling time may be required. Please note that the yield of recovery for smaller antibody amounts may be diminished.
Do I need to purify my labeled antibody?	It is strongly recommended to purify the labeled antibody away from the excess label and Sortase A5 prior to use. If the antibody will be used immediately, skipping purification may be acceptable. However, storage of unpurified antibody over time will result in removal of the label. Any active Sortase A5 will continue to cleave at the recognition sequence and may create large antibody complexes or completely remove the poly-Glycine labels resulting in unlabeled antibody. Therefore, purification prior to use and storage is strongly recommended.

Technical Services

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