Sortag-IT™ ATTO 488 Labeling Kit

Catalog No.: 13107
Format: 3 x 100 µg

Description

The Sortag-IT™ ATTO 488 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.

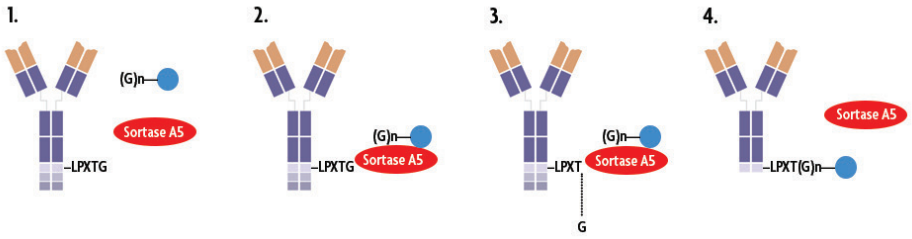


Figure 1: The Sortag-IT™ antibody labeling system.

(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.

Contents

Each Sortag-IT™ ATTO 488 Labeling Kit provides sufficient materials to label 3 x 100 µg AbFlex™ recombinant antibody with a yield > 50%.

- 3 units Sortase A5 enzyme (1 unit/µl). Store at -80°C
- 1 ml Reaction Buffer AM3; Store at 4°C
- 1.53 mM $(Gly)_5$ - ATTO 488 label (1.53 mM = 2.47 mg/ml); 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 ATTO 488:
Reaction conditions are label-specific.

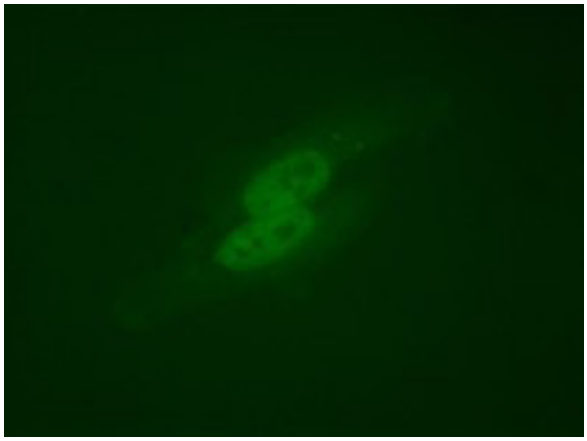
1. Thaw the vial of Sortase A5 enzyme on ice.
2. Set up the labeling reactions in a 1.5 ml microcentrifuge tube. Keep tube wrapped in foil and protected by light during the labeling reaction. Add reagents in the order shown below and mix by pipetting.

Reagent	Volume to add
AbFlex antibody (1 µg/µl)	100 µl
Reaction Buffer AM3	83 µl
$(Gly)_5$-ATTO label (1.53 mM)	16 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. Keep sample protected from light during incubation.
4. Following the incubation, remove excess label from the reaction by purification using the included columns. Keep samples protected from light as much as possible.
   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. Wrap the new collection tube in foil and protect from light.
5. Add 2.5 µl Stop Solution AM3 to each labeled antibody to inactivate the Sortase A5 enzyme.
6. Use immediately, or store in the dark at 4°C for up to 3 months.

Appendix

Application Data



Immunofluorescence stain of Histone H3K9ac-ATTO 488 directly conjugated antibody.

The Sortag-IT™ ATTO 488 Labeling Kit was used to directly conjugate 100 µg of Active Motif's AbFlex™ Histone H3K9ac recombinant antibody with ATTO 488. Staining shows nuclear localization of the Histone H3K9ac antibody as expected.

Troubleshooting

Problem/question	Recommendation
What is the excitation range for ATTO 488?	ATTO 488 fluorescence is excited most efficiently in the range of 480-515 nm.
How can I determine the labeling efficiency?	For ATTO 488 the Degree of Labeling (DOL) can be determined using Lambert-Beer law. 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). We anticipate a DOL value greater than 1.0 $DOL = \frac{A_{max} / E_{max}}{A_{prot} / E_{prot}} = \frac{A_{max} \times E_{prot}}{(A_{280} - A_{max} \times CF) \times E_{max}}$ A_{280} = absorbance of conjugate solution measured at 280 nm. A_{max} = absorbance of conjugate solution measured at λEx (500 nm for ATTO 488) CF = correction factor = A_{280}/A_{max} (0.1 for ATTO 488) E_{prot} = extinction coefficeint for antibody (210,000 M-1cm-1 for IgG) E_{max} = extinction coefficeint for ATTO dye (90,000 M-1cm-1 for ATTO 488)
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

If you need assistance at any time, please call Active Motif Technical Service at one of the numbers listed below.

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