ACTIVE MOTIF®

Enabling Epigenetics Research

# Sortag-IT™ Azide Click Labeling Kit

Catalog No. 13114

(Version A1)

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## Overview

The Sortag-IT™ 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 which has activity >15 times wild-type Sortase, allowing for a faster, more efficient labeling reaction. Each antibody contains two Sortase tag sequences.

The Sortag-IT™ conjugation system is site directed, preventing non-specific conjugation that might interfere with antibody binding and provides control over how many conjugates are attached per antibody. Active Motif's latest advance in sortase mediated conjugation incorporates click chemistry, resulting in simplified conjugations and more conjugates per antibody.

| Product | Format | Catalog No. |
|---|---|---|
| Sortag-IT™ Azide Click Labeling Kit | 3 x 100 µg | 13114 |


*US Patent 9,267,127

## Process Overview

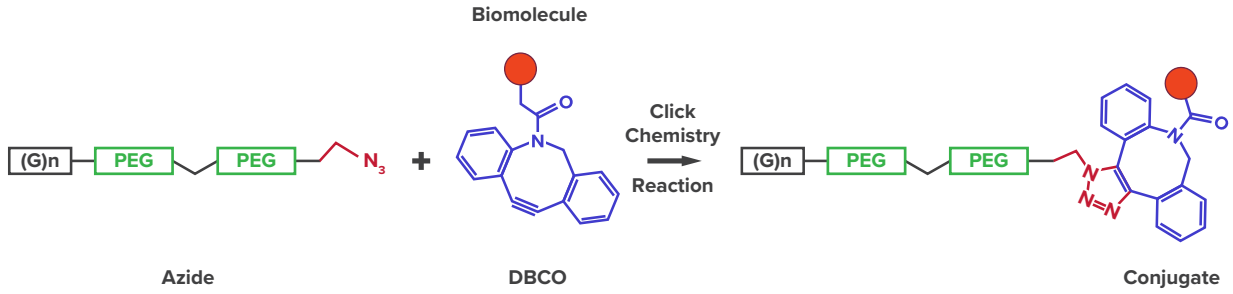



**Figure 1: Click Chemistry Reaction Schematic.**
Click chemistry works through the presence of an Azide group on the donor molecule and a DBCO group on the acceptor molecule. Covalent attachment is achieved by simply mixing the two components.

The new Sortag-IT™ linkers provide even more control over the number of conjugates added per antibody and are compatible with any number of commercially available click chemistry conjugates such as dyes or oligos. Click chemistry reactions are fast, simple to use, versatile, and give high product yields. First, Sortase is used to attach the linker to the Sortase recognition sequence in the AbFlex® antibody (**Figure 2**). Then click chemistry can be used to add DBCO containing molecules to the AbFlex® - Azide acceptor molecule (**Figure 3**). Labeling of both heavy chains using this linker results in 2 conjugation sites per antibody.

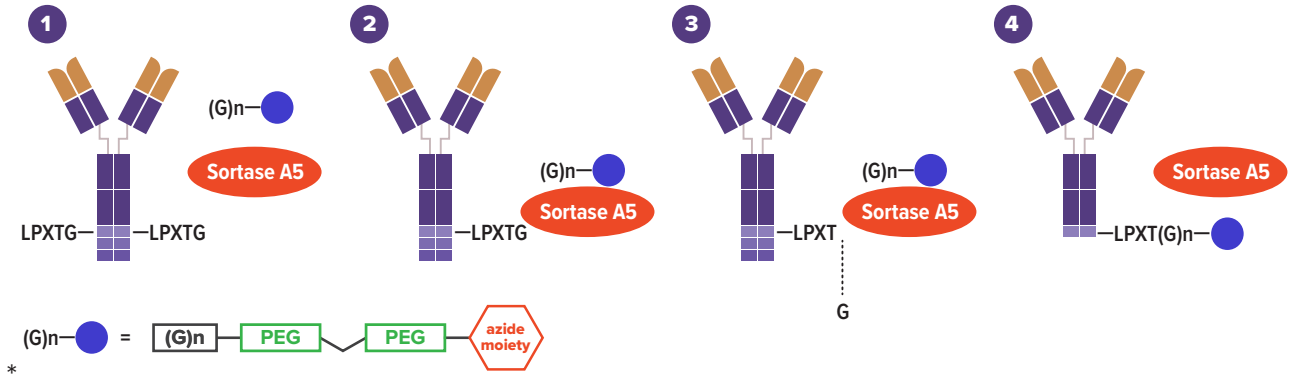



**Figure 2: Sortag-IT™ Azide Click Labeling Kit and Sortase A5 Labeling.**
(1) Combine the buffer exchanged AbFlex® recombinant antibody (rAb) of interest in Reaction Buffer with the (Gly)5- Azide Click label at a ratio of greater than or equal to 5 nmol label to 1 nmol antibody in the presence of Sortase A5 enzyme. (2) Sortase A5 will bring the (Gly)5- Azide Click 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 (Gly)5- Azide Click 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. This results in an antibody containing an Azide group for use in click chemistry.

* This kit provides a poly-Glycine label with one Azide moiety as Click Chemistry component (Gly)5- Azide Click label).

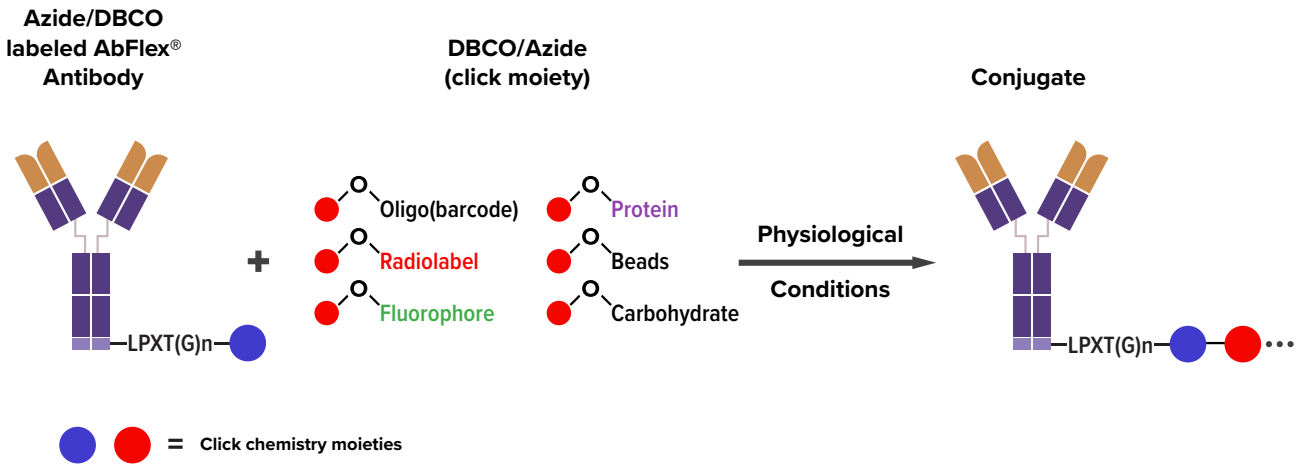



**Figure 3: The Sortag-IT™ Azide Click Labeled AbFlex® Antibody Used in Click Chemistry Reactions.**
The labeled AbFlex® antibody containing an Azide click chemistry groups at the C-terminus can be reacted with a series of DBCO containing molecules resulting in a click chemistry mediated comjugation.

## Kit Components and Storage

Please store each component at the temperature indicated in the table below. Each Sortag-IT™ Azide Labeling Kit provides sufficient materials to label 3 x 100 µg AbFlex® recombinant antibody with a yield > 60%.

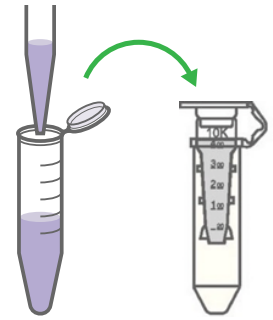
| Kit Component | Quantity | Storage |
|---|---|---|
| Sortase A5 enzyme | 12 units (3 tubes) | -80°C |
| Reaction Buffer AM3 | 3.2 mL (3 tubes) | 4°C |
| (Gly)5- Azide Click label | 24 µL | -20°C |
| Stop Solution AM3 | 8 µL | RT |
| Purification columns | 6 columns (3 per bag) | RT |

## Additional Materials Required

- 100 µg AbFlex® Recombinant antibody per reaction (for a complete product listing visit activemotif.com/abflex)
- 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 $KH_2PO_4$)

### Buffer Exchange

1. Add 250 µL of Reaction Buffer AM3 to the AbFlex® antibody tube and transfer to the included column.




2. Spin the column at 13,500 x *g* for 5 minutes in a microcentrifuge. Discard flow through and replace column in the collection tube.
3. Add 400 µL of Reaction Buffer AM3 to the column/collection tube assembly and spin the column at 13,500 x *g* for 5 minutes. Discard flow through.
4. Add 150 µL Reaction Buffer AM3 to the column. Place the column inverted into a new microcentrifuge tube.
5. Spin column at 800 x *g* for 2 minutes. Save the flow through and discard the column. The flow through contains your buffer exchanged antibody.
6. Measure the volume and add Reaction Buffer AM3 to achieve a total of 250 µL.

### Labeling

7. Thaw the vial of Sortase A5 enzyme on ice.
8. Set up the labeling reaction by adding the reagents in the order shown in the table below and mix by pipetting.

| Kit Component | Volume to add |
|---|---|
| AbFlex® antibody in Reaction Buffer AM3 | 250 µL |
| (Gly)5- Azide Click label (5 mM) | 8 µL |
| Sortase A5 enzyme (1 unit/µL) | 4 µL |

9. Incubate the labeling reaction at 30°C for 1 hour with shaking at 1000 rpm using a Thermomixer or similar device.

**Label Clean-Up**

10. Following the incubation, remove excess label from the reaction by purifying with the included column.
    a. Add 200 µL of PBS to the antibody labeling reaction.
    b. Add the entire reaction to the column.
    c. Spin column at 13,500 x *g* for 5 minutes in a microcentrifuge. Discard flow through and replace the column in a new collection tube.
    d. Wash the column by adding 400 µL PBS then spin column at 13,500 x *g* for 5 minutes in a microcentrifuge. Discard the flow through and replace the column in the collection tube.
    e. Repeat the wash from **step d** for two additional times for a total of three washes.
    f. Add 100 µL PBS to the column. Place the column inverted into a new microcentrifuge tube.
    g. Spin column at 800 x *g* for 2 minutes to collect the labeled antibody.
11. Add 2.5 µL Stop Solution AM3 to labeled antibody to inactivate the Sortase A5 enzyme. Invert to mix
12. Use immediately for click conjugation, or store at 4°C for up to 1 month.

**Sortase Site Directed Click Chemistry Reaction (not included in the kit):**

13. Following the lab clean-up proceed to use the Azide labeled AbFlex® antibody for click chemistry.
14. Set up the click chemistry reaction by adding 400 pmol of DBCO biomolecule to 20 pmol of Azide labeled AbFlex® antibody (labeled in this kit).
15. Incubate the reaction at 4°C for 24 hours then remove excess label using size exclusion or purification of choice.

# Appendix

## Application Data

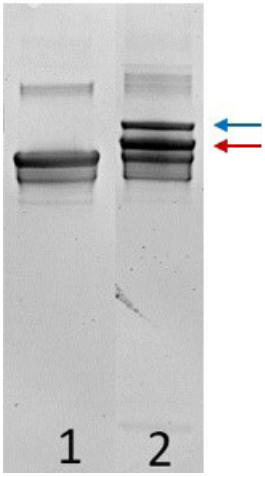



**Figure 4: AbFlex® H3K27ac Antibody (rAb) Conjugated to DNA Using Active Motif's Azide Click Label**
AbFlex® H3K27ac antibody (rAb) (Cat No. 91193) was first labeled with Sortag-IT™ Azide Click Labeling Kit (Cat No. 13114). Then 20 pmol of azide labeled antibody was mixed with 400 pmol of 70-mer oligonucleotide bearing a DBCO* group and incubated at 4°C overnight. The samples were loaded onto a 4-12% SDS-PAGE and stained with Coomassie blue.

Lane 1: AbFlex® H3K27ac unlabeled control antibody.

Lane 2: AbFlex® H3K27ac Azide antibody (rAb) conjugated to a 70-mer oligonucleotide.

The red arrow shows the antibody with 1 oligonucleotide conjugated.

The blue arrow shows the antibody with 2 oligonucleotides conjugated.

*DBCO-modified oligonucleotide was prepared from commercial amine-modified oligonucleotide. Commercial amine-modified (3' or 5') oligonucleotide was dissolved in water (0.3-0.5 mM; 60 µL) and precipitated in pre-chilled (-20°C) 3% lithium perchlorate in acetone (1.7 mL). The pellet was then washed with acetone (2 x 0.7 mL) and dried. The oligonucleotide pellet was dissolved in 60 µL 0.1M MOPS buffer (pH 7.7). DBCO-PEG-NHS (9 µL; 25 mg/mL in DMF) was added and the reaction mixture was incubated for 2 hr followed by precipitation in pre-chilled (-20°C) 3% lithium perchlorate in acetone (1.7 mL). The pellet was washed with acetone (2 x 0.7 mL) then dried. DBCO modified oligonucleotide was used for the conjugation without additional purification.

DBCO-PEG4-NHS Ester (Cat No. A134, Click Chemistry Tools)

# Troubleshooting Guide

| Problem/Question | Recommendation |
|---|---|
| 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 and reaction yield may prohibit the addition of 2 labels per antibody molecule).<br>We recommend performing a click reaction with DBCO-modified oligonucleotide (40-70-mer) followed by SDS-PAGE as described in Application Data section.<br>A commercially available dye modified with DBCO can be used. After performing the “click” reaction, dye/protein ratio can be determined according to the dye manufacturer protocol. |
| 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 modiffied in the reaction. Volume differences should be corrected using Reaction Buffer AM3 to maintain a total volume of 262 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 purifica- tion 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 or send an e-mail to Active Motif Technical Service at one of the locations listed below.

**North America**

| | |
|---|---|
| Toll free: | 877 222 9543 |
| Direct: | 760 431 1263 |
| Fax: | 760 431 1351 |
| E-mail: | tech_service@activemotif.com |

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