

# SiteClick™ Antibody Azido Modification Kit

Catalog No. S20026

Pub. No. MAN0017078

Rev. A.0

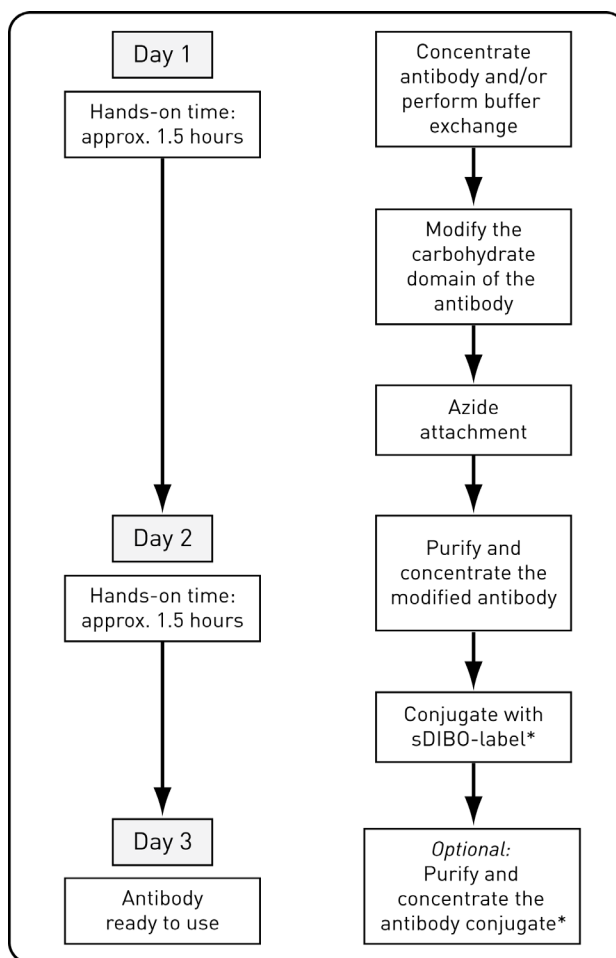
## Product description

The SiteClick™ Antibody Azido Modification Kit allows you to specifically attach an azide moiety to the heavy chains of an unlabeled IgG antibody, ensuring that the antigen binding domains of the antibody remain unaltered for binding to your antigen target. The azide-modified antibody can then be covalently linked to Click-iT™ sDIBO Alkyne labels (available separately; see Table 2, page 2) in a copper-free click reaction without reducing the protein. This gives you the option to choose different fluorescent labels for your antibody, attach another molecule via streptavidin, or attach your own molecule via amine-reactive or amine-containing moieties depending on your assay.

Each SiteClick™ Antibody Azido Modification Kit contains sufficient reagents to perform one azido modification reaction starting with 100–250 µg of whole IgG from any host species produced in eukaryotic cells. The antibody concentrators provided in the kit are used to purify and concentrate the antibody at each step of the SiteClick™ antibody labeling workflow (Figure 1, page 2)

**Table 1** Contents and storage

Material	Amount	Storage*
Antibody preparation buffer (Component A)	1.8 mL	<ul style="list-style-type: none"><li>• 2–8°C</li><li>• DO NOT FREEZE</li></ul>
Antibody concentrator (small) (Component B)	each	
Collection tube (Component C)	each	
β-Galactosidase (Component D)	12 μL	
UDP-GalNAz (Component E)	220 μg	
20X Tris pH 7.0 (1M) (Component F)	1.8 mL	
Buffer additive (Component G)	30 μL	
β-1,4-galactosyltransferase (GalT) (Component H)	88 μL	
Antibody concentrator (large) (Component I)	2 each	
* When stored as directed, this kit is stable for at least 6 months.		



\* Requires the use of the Click-iT™ sDIBO Alkyne for SiteClick Antibody Labeling kits (available separately).

**Figure 1** SiteClick™ antibody azido modification and antibody labeling workflow. The SiteClick™ Antibody Azido Modification Kit is designed to be used with the Click-iT™ sDIBO Alkyne for SiteClick™ Antibody Labeling kits (available separately; see Table 2) for a complete antibody labeling workflow.

**Table 2** Click-iT™ sDIBO Alkynes for SiteClick™ Antibody Labeling. The Click-iT™ sDIBO Alkyne labels (available separately) are used in conjunction with the SiteClick™ Antibody Azido Modification Kit or with engineered antibodies containing azido moieties to create high-quality antibody conjugates.

Product	Catalog No.
Click-iT™ Alexa Fluor™ 488 sDIBO Alkyne	C20027
Click-iT™ Alexa Fluor™ 555 sDIBO Alkyne	C20028
Click-iT™ Alexa Fluor™ 647 sDIBO Alkyne	C20029
Click-iT™ Biotin sDIBO Alkyne	C20030
Click-iT™ Amine sDIBO Alkyne	C20031
Click-iT™ SDP Ester sDIBO Alkyne	C20032

## Before you begin

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Equipment required	<ul style="list-style-type: none"><li>• Centrifuge with fixed angle rotor that can accommodate 1.5-mL centrifuge tubes</li><li>• Centrifuge with swinging bucket rotor that can accommodate 17 mm × 100 mm centrifuge tubes</li></ul>
Materials required but not provided	<ul style="list-style-type: none"><li>• 100–250 µg of whole IgG antibody produced in eukaryotic cells, preferably at a concentration of 2–20 mg/mL in a Tris-based buffer, free of carrier proteins and/or azide</li><li>• Centrifuge tubes: 1.5-mL and 15-mL</li><li>• Distilled water (dH<sub>2</sub>O)</li><li>• PBS or TBS</li><li>• Click-iT™ sDIBO Alkyne label (sDIBO-dye, sDIBO-biotin, or sDIBO-chelator) (Table 2, page 2)</li></ul>
Caution	<ul style="list-style-type: none"><li>• <b>IMPORTANT!</b> Sodium azide must be avoided throughout the protocol.</li><li>• β-Galactosidase (Component D) may cause an allergic skin reaction, and it may cause allergy or asthma symptoms or breathing difficulties, if inhaled. Read the Safety Data Sheet (SDS), available at <a href="http://thermofisher.com">thermofisher.com</a>, before handling this reagent.</li><li>• Dispose of the reagents in compliance with all pertaining local regulations. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Always wear suitable laboratory protective clothing and gloves when handling these reagents.</li></ul>

## Step 1. Concentrate antibody and/or perform buffer exchange (optional)

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**Time required:** 1 hour

This antibody concentration and buffer exchange step is required if:

- Your antibody concentration is less than 2 mg/mL, and/or
- Your antibody is in a phosphate-based buffer (e.g. PBS), and/or
- Your antibody is in a buffer containing azide.

Before you begin, briefly centrifuge the tubes containing enzymes, substrates, or dyes to ensure all material is at the bottom of the tubes.

### Wash the antibody concentrator

- 1.1 Add 500 µL of dH<sub>2</sub>O to the small antibody concentrator (Component B) and cap the device as shown in Figure 2 (page 4).
- 1.2 Centrifuge at 5000 × g for 6 minutes, ensuring that the cap strap and one membrane panel of the concentrator faces the center of the rotor.
- 1.3 Discard the flow-through.

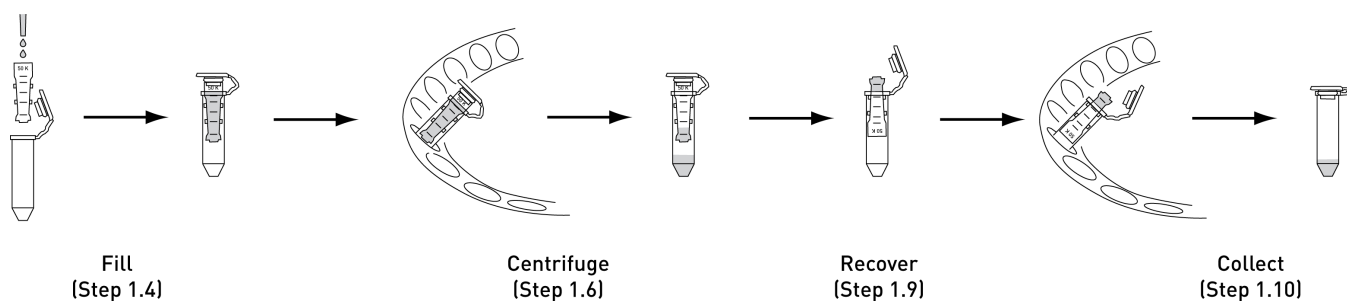


Figure 2 Antibody concentration and/or buffer exchange

### Concentrate antibody and/or perform buffer exchange

- 1.4 Add a sufficient volume of antibody solution to contain 100–300  $\mu\text{g}$  of antibody to the small antibody concentrator.
  - 1.5 Dilute the added antibody to 500  $\mu\text{L}$  using antibody preparation buffer (Component A).
  - 1.6 Centrifuge at  $5000 \times g$  for 6 minutes, ensuring that the cap strap and one membrane panel of the concentrator faces the center of the rotor.
  - 1.7 Discard the flow-through.
  - 1.8 Add 450  $\mu\text{L}$  of antibody preparation buffer (Component A) to the small antibody concentrator (Component B) and centrifuge at  $5000 \times g$  for 6 minutes, ensuring that the cap strap and one membrane panel of the concentrator faces the center of the rotor.
- Note:** If antibody volume in concentrator is greater than 50  $\mu\text{L}$  following Step 1.8, centrifuge at  $5000 \times g$  for an additional 3 minutes or until the appropriate volume is achieved.
- 1.9 Invert the small antibody concentrator (Component B) into the collection tube (Component C) as shown in Figure 2.
  - 1.10 Centrifuge for 3 minutes at  $1000 \times g$  to collect the concentrated antibody. Following collection, you should have approximately 50  $\mu\text{L}$  of concentrated antibody in the collection tube.

## Step 2. Modify the carbohydrate domain of the antibody

**Time required:** 5 minutes hands-on, then 6 hours incubation

### Add $\beta$ -galactosidase

- 2.1 Add 10  $\mu$ L of  $\beta$ -galactosidase (Component D) to the antibody collected in Step 1.10, as shown in Figure 3.
- 2.2 Wrap the tube cap with Parafilm™ laboratory film or similar, then incubate for 6 hours to overnight at 37°C.

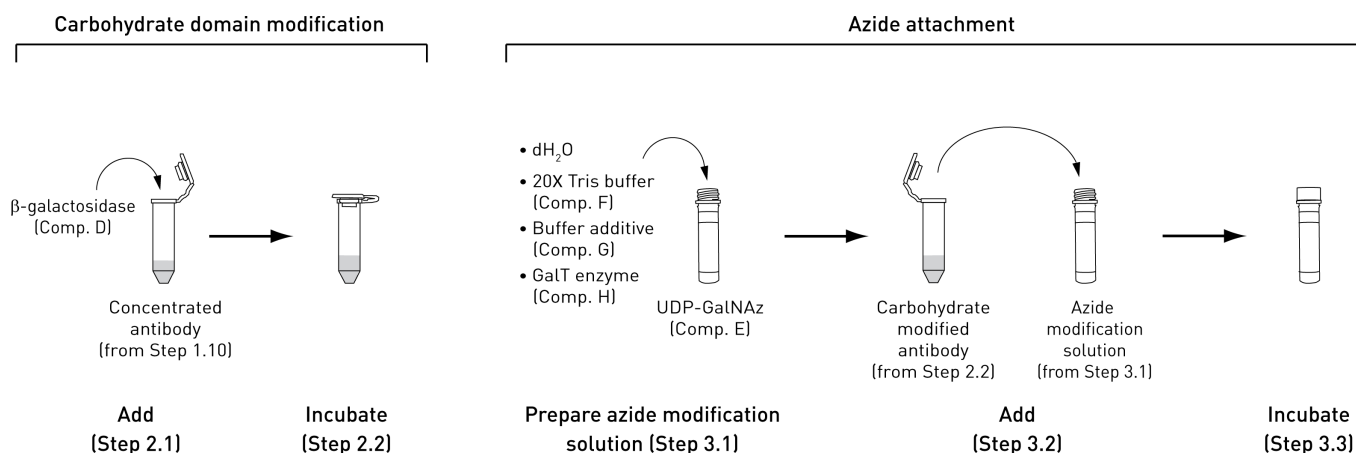


Figure 3 Modification of antibody carbohydrate domain and azide attachment

## Step 3. Azide attachment

**Time required:** 5 minutes hands-on, then overnight incubation

### Add GalT enzyme

- 3.1 Prepare the azide modification solution by adding the following components to the tube containing UDP-GalNAz (Component E), as shown in Figure 3:
  - 75  $\mu$ L of dH<sub>2</sub>O
  - 12.5  $\mu$ L of 20X Tris buffer, pH 7.0 (Component F)
  - 25  $\mu$ L of buffer additive (Component G)
  - 80  $\mu$ L of GalT enzyme (Component H)
- 3.2 Vortex the reaction components and then add the modified antibody from Step 2.2 to the tube.
- 3.3 Briefly centrifuge the tube, wrap the tube cap with Parafilm™ laboratory film or similar, then incubate overnight at 30°C.

## Step 4. Purify and concentrate the azide-modified antibody

**Time required:** 1 hour

- This step removes any excess substrate UDP-GalNAz.
- You may also use TBS or other phosphate free buffers for purification and collection of the modified antibody (Steps 4.2–4.12). 20X Tris, pH 7.0 is provided for your convenience.

**4.1** Prepare 10 mL of 1X Tris, pH 7.0 by adding 500  $\mu$ L of 20X Tris, pH 7.0 (Component F) to 9.5 mL of dH<sub>2</sub>O in a 15-mL conical tube. Vortex briefly to mix.

### Wash the antibody concentrator

**4.2** Remove the conical collection tube from the large antibody concentrator (Component I).

**4.3** Add 1 mL of 1X Tris, pH 7.0 (or TBS) to the large antibody concentrator (Component I) as shown in Figure 4.

**4.4** Centrifuge at  $1200 \times g$  for 10 minutes, ensuring that one membrane panel of the concentrator faces the center of the rotor. Discard the flow-through.

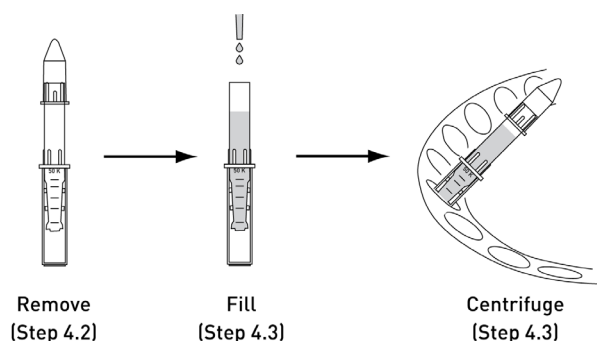


Figure 4 Wash the antibody concentrator

### Purify the antibody

**4.5** Add 1.6 mL of 1X Tris pH 7.0 (or TBS) and 250  $\mu$ L of the azide-modified antibody from Step 3.3 to the large antibody concentrator (Component I) as shown in Figure 5.

**4.6** Centrifuge at  $1200 \times g$  for 6 minutes, ensuring one membrane panel of the concentrator faces the center of the rotor. Discard the flow-through.

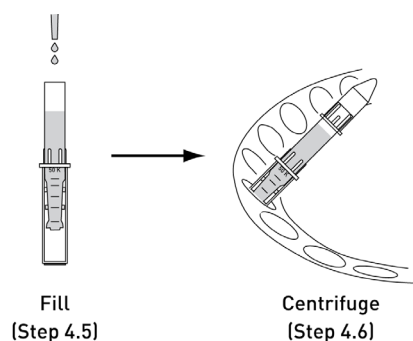


Figure 5 Purification and concentration of azide-modified antibody

4.7 Add 1X Tris pH 7.0 (or TBS) to a total volume of 2 mL to the large antibody concentrator (Component I) and centrifuge at  $1200 \times g$  for 10 minutes, ensuring that one membrane panel of the concentrator faces the center of the rotor.

4.8 Discard the flow-through and repeat Step 4.7 two more times.

**Note:** If the antibody volume in the concentrator is greater than  $\sim 100 \mu\text{L}$  or an antibody concentration of more than  $\sim 2.0 \text{ mg/mL}$  is desired, you can reduce the volume in the concentrator by additional centrifugation (e.g., at  $1200 \times g$  for an additional 5 minutes or until the appropriate volume is achieved).

#### Collect the azide-modified antibody

4.9 Invert the antibody concentrator into the conical collection tube as shown in Figure 6.

4.10 Centrifuge at  $1000 \times g$  for 3 minutes to collect the concentrated antibody.

4.11 Transfer the antibody from the conical collection tube to a 1.5-mL centrifuge tube.

4.12 Determine the antibody concentration by measuring  $\text{OD}_{280}$  (with  $\text{OD}_{280}$  at 1.4 = 1 mg/mL). Expected concentration is  $\sim 1\text{--}5 \text{ mg/mL}$ .

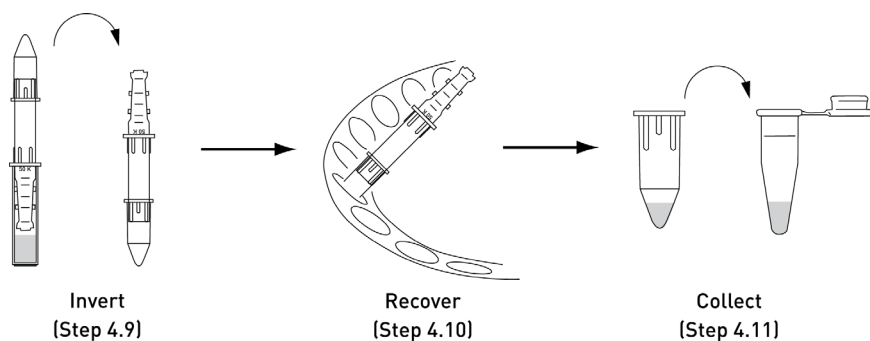


Figure 6 Collection of purified and concentrated azide-modified antibody

#### Store the azide-modified antibody

At this point, you can store the azide-modified antibody at  $2\text{--}8^\circ\text{C}$  until needed. Do not freeze the azide-modified antibody.

**IMPORTANT!** If you wish to perform a click reaction to conjugate your azide-modified antibody to a Click-iT<sup>™</sup> sDIBO Alkyne label, do not add sodium azide to your modified antibody. Sodium azide must be avoided throughout the protocol.

## Step 5. Attach sDIBO-modified label to azide-modified antibody

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**Time required:** 5 minutes hands-on, then overnight incubation

This section provides instructions to covalently link the azide-modified antibody to a Click-iT™ sDIBO Alkyne label in a copper-free click reaction.

### Materials required but not provided

- Azide-modified antibody (from Step 4.11) in a Tris-based buffer, free of carrier proteins and/or azide
- Click-iT™ sDIBO Alkyne label (available separately; see Table 2, page 2)
- Anhydrous DMSO (only required for the resuspension of Click-iT™ SDP Ester sDIBO Alkyne; included in Cat. No. C20032)
- Distilled water (dH<sub>2</sub>O)
- PBS or TBS
- 1.5-mL centrifuge tubes

### Caution

- **IMPORTANT!** Sodium azide must be avoided throughout the protocol.
- DMSO is known to facilitate the entry of organic molecules into tissues. Handle reagents containing DMSO using equipment and practices appropriate for the hazards posed by such materials.
- Read the Safety Data Sheet (SDS), available at [thermofisher.com](http://thermofisher.com), before handling the reagents.
- Dispose of the reagents in compliance with all pertaining local regulations. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Always wear suitable laboratory protective clothing and gloves when handling these reagents.

### Add Click-iT™ sDIBO Alkyne to azide-modified antibody

- 5.1** Add 11 µL of Click-iT™ sDIBO Alkyne label to 100 µg azide-modified antibody in 100 µL of 1X Tris pH 7.0 (or TBS) in the 1.5-mL centrifuge tube.

**Note:** The Click-iT™ SDP Ester sDIBO Alkyne for SiteClick™ Antibody Labeling (Cat. No. C20032) is supplied lyophilized as a solid powder. Before use, resuspend the Click-iT™ SDP Ester sDIBO Alkyne in 25 µL of anhydrous DMSO, which is included in the kit.

Other Click-iT™ sDIBO Alkynes for SiteClick™ Antibody Labeling are supplied as 25-µL solutions in DMSO and do not need to be resuspended.

- 5.2** Vortex the reaction mixture, briefly centrifuge, and incubate overnight at 25°C.

**Note:** Following incubation, you can store the antibody conjugate at 2–8°C until needed (see “Store the antibody conjugate”, page 10) or purify it of the excess unconjugated antibody (Step 6, optional).



## Step 6. Purify and concentrate the antibody conjugate (optional)

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**Time required:** 1 hour

- The purification step removes any excess antibody that has not been conjugated with the sDIBO-modified label.
- You may use TBS or PBS for the purification and collection of the modified antibody (Steps 6.2–6.7)

### Materials required but not provided

- Antibody conjugate (from Step 5.2)
- Antibody concentrator, large (included in the Click-iT™ sDIBO Alkyne for SiteClick™ Antibody Labeling kits)  
**Note:** The antibody concentrator included in the Click-iT™ sDIBO Alkyne kits (Component B; Component C in Cat. No. C20032) is identical to the large antibody concentrator (Component I) supplied with the SiteClick™ Antibody Azido Modification Kit.
- Distilled water (dH<sub>2</sub>O)
- PBS or TBS
- 1.5-mL centrifuge tubes

### Wash the antibody concentrator

- 6.1 Remove the conical collection tube from the large antibody concentrator.
- 6.2 Add 1X Tris, TBS, or PBS to a total volume of 2 mL to the large antibody concentrator (Component I) as shown in Figure 4 (page 6).
- 6.3 Centrifuge at 1200 × g for 10 minutes, ensuring that one membrane panel of the concentrator faces the center of the rotor. Discard the flow-through.

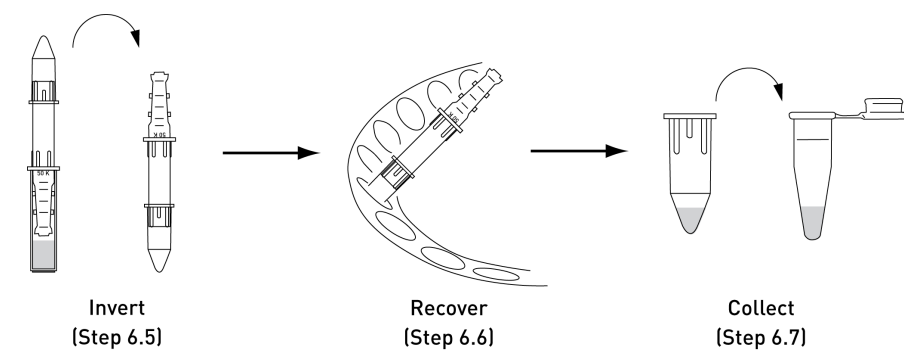
### Purify the antibody conjugate

- 6.4 Add 1.6 mL of 1X Tris, TBS, or PBS and 200 µL of the sDIBO-modified antibody (from Step 5.2) to the large antibody concentrator.
- 6.2 Centrifuge at 1200 × g for 10 minutes, ensuring that one membrane panel of the concentrator faces the center of the rotor. Discard the flow-through.
- 6.3 Add 1.8 mL of 1X Tris, TBS, or PBS to the large antibody concentrator (Component I), then centrifuge at 1200 × g for 10 minutes, ensuring that one membrane panel of the concentrator faces the center of the rotor.
- 6.4 Discard the flow-through and repeat Step 6.3 two more times.

**Note:** If an antibody concentration of more than ~0.5 mg/mL is desired, you can reduce the volume in the concentrator by additional centrifugation (e.g., at 1200 × g for an additional 5 minutes or until the appropriate volume is achieved).

**Collect the purified antibody conjugate**

- 6.5** Invert the antibody concentrator into the conical collection tube as shown in Figure 7.
- 6.6** Centrifuge at  $1000 \times g$  for 3 minutes to collect the concentrated antibody.
- 6.7** Transfer the purified antibody conjugate from the conical collection tube to a new 1.5-mL centrifuge tube.



**Figure 7** Optional purification and concentration of the labeled antibody conjugate

**Store the antibody conjugate**

Store the antibody conjugate at 2–8°C until needed. **DO NOT FREEZE.**

You can add sodium azide or thimerosal at this stage to a final concentration of 0.02% (w/v) for long term storage, if preferred.

## Appendix: Assay principle

In the first step of SiteClick™ conjugation, terminal galactose residues on the N-linked sugars in the Fc region of the antibody are removed by  $\beta$ -Galactosidase. The azide-containing sugar, GalNAz, is then added to the modified carbohydrate domain of the antibody via the  $\beta$ -1,4-galactosyltransferase (Gal-T)-catalyzed reaction targeting the terminal GlcNAc residues. This specific targeting maintains the integrity of the antigen binding site on the antibody. Finally, the antibody (now containing an azide moiety) is conjugated to the sDIBO-modified label in a copper-free click reaction with simple overnight incubation (Figure 8).

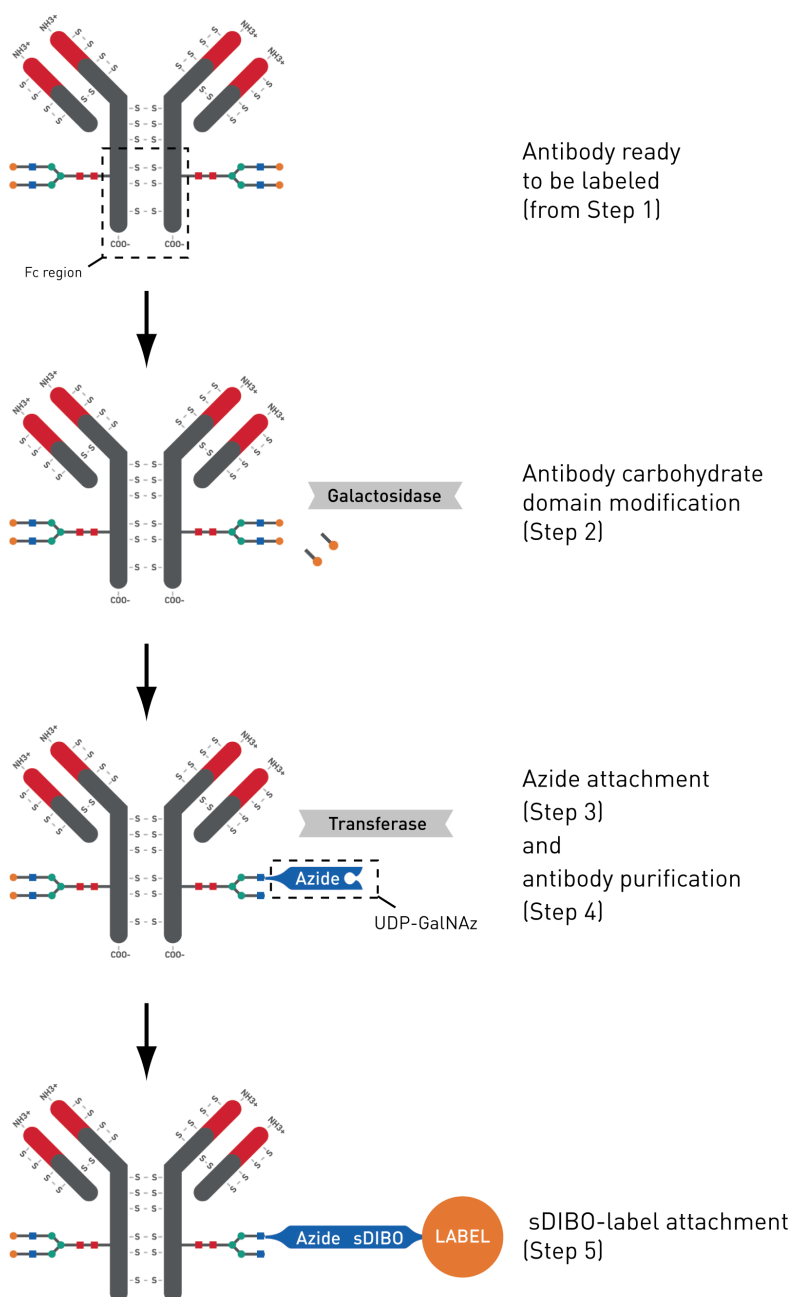


Figure 8 SiteClick™ conjugation reaction

## Ordering information

Cat. No.	Product name	Unit size
S20026	SiteClick™ Antibody Azido Modification Kit. . . . .	1 kit

### Related products

C20027	Click-iT™ Alexa Fluor™ 488 sDIBO Alkyne for SiteClick™ Antibody Labeling. . . . .	1 kit
C20028	Click-iT™ Alexa Fluor™ 555 sDIBO Alkyne for SiteClick™ Antibody Labeling. . . . .	1 kit
C20029	Click-iT™ Alexa Fluor™ 647 sDIBO Alkyne for SiteClick™ Antibody Labeling. . . . .	1 kit
C20030	Click-iT™ Biotin sDIBO Alkyne for SiteClick™ Antibody Labeling . . . . .	1 kit
C20031	Click-iT™ Amine sDIBO Alkyne for SiteClick™ Antibody Labeling . . . . .	1 kit
C20032	Click-iT™ SDP Ester sDIBO Alkyne for SiteClick™ Antibody Labeling . . . . .	1 kit
S20033	SiteClick™ Biotin Antibody Labeling Kit . . . . .	1 kit

## Purchaser notification

These high-quality reagents and materials must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Read the Safety Data Sheet provided for each product; other regulatory considerations may apply.

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For the latest services and support information for all locations, go to [thermofisher.com/support](http://thermofisher.com/support).

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- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support ([thermofisher.com/support](http://thermofisher.com/support))
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

### SDS

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**Revision history:** Pub. No. MAN0017078

Revision	Date	Description
A.0	11 May 2017	New User Guide

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