SiteClick™ Biotin Antibody Labeling Kit

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Pub. No. MAN0017077 **Rev.** A.0

Product description

The SiteClick™ Biotin Antibody Labeling Kit allows you to conjugate your own antibodies to sDIBO-modified biotin. The SiteClick™ conjugation workflow consists of three steps (antibody carbohydrate domain modification, azide attachment to the antibody, and conjugation with the sDIBO-modified biotin) and relies on copper-free click chemistry to covalently link the biotin containing the sDIBO moiety with the azide-modified antibody without reducing the protein. 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).

Each SiteClick™ Biotin Antibody Labeling Kit contains sufficient reagents to perform one conjugation reaction of sDIBO-modified biotin to a primary IgG antibody sample from any host species produced in eukaryotic cells. This user guide describes a SiteClick™ biotin labeling workflow starting with 100–250 µg of whole IgG.

Table 1 Contents and storage

Material	Amount	Storage*
Antibody preparation buffer (Component A)	1.8 mL	• 2–8°C • DO NOT FREEZE
Antibody concentrator (small) (Component B)	each	
Collection tube (Component C)	each	
ß-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	
ß-1,4-galactosyltransferase (GalT) (Component H)	88 µL	
Antibody concentrator (large) (Component I)	2 each	
Click-iT™ Biotin sDIBO alkyne (Component J)	25 µL	
* When stored as directed, this kit is stable for at least 6 months.		

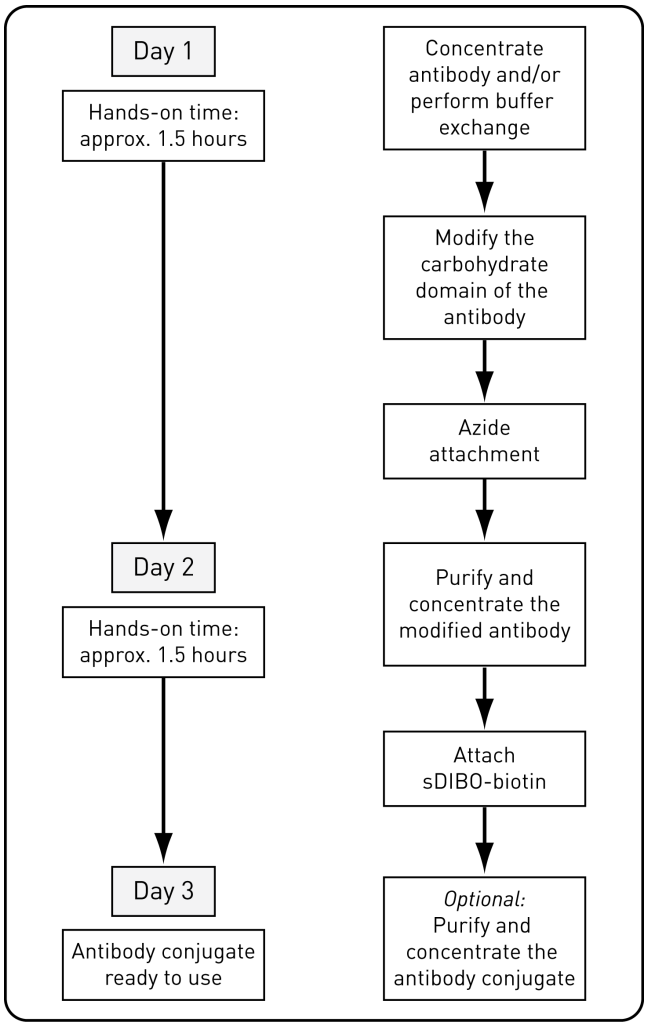

Figure 1 SiteClick™ antibody labeling workflow

Before you begin

Equipment required

- Centrifuge with fixed angle rotor that can accommodate 1.5-mL centrifuge tubes
- Centrifuge with swinging bucket rotor that can accommodate 17 mm × 100 mm centrifuge tubes

Materials required but not provided

- 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
- Centrifuge tubes: 1.5-mL and 15-mL
- Distilled water (dH_2O)
- PBS or TBS

Caution

- **IMPORTANT!** Sodium azide must be avoided throughout the protocol.
- 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 **thermofisher.com**, before handling this reagent.
- 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.

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

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_2O 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.

Concentrate antibody and/or perform buffer exchange

1.4 Add a sufficient volume of antibody solution to contain 100–300 µg of antibody to the small antibody concentrator.

1.5 Dilute the added antibody to 500 µL using antibody preparation buffer (Component A).

1.6 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.

1.7 Discard the flow-through.

1.8 Add 450 µL of antibody preparation buffer (Component A) to the small antibody concentrator (Component B) and 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.

Note: If antibody volume in concentrator is greater than 50 µL following Step 1.8, centrifuge at 5000 × *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 × *g* to collect the concentrated antibody. Following collection, you should have approximately 50 µL of concentrated antibody in the collection tube.

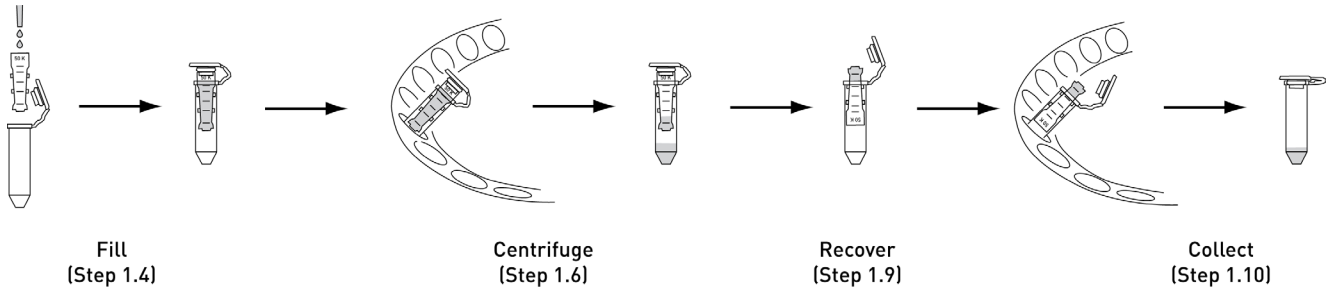

Figure 2 Antibody concentration and/or buffer exchange

Step 2. Modify the carbohydrate domain of the antibody

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

Add ß-galactosidase

2.1 Add 10 µL of β-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.

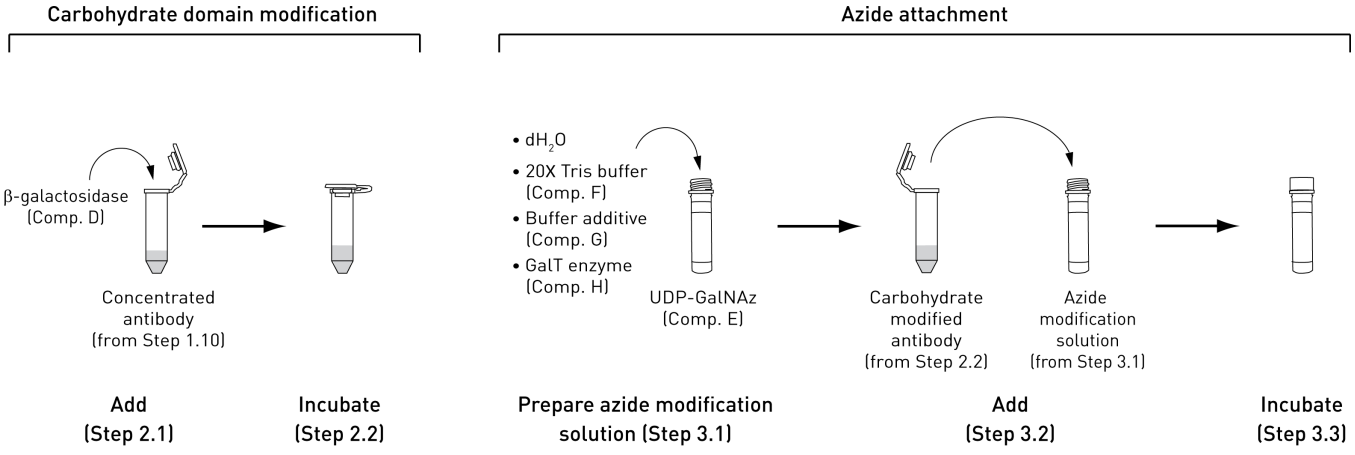

Figure 3 Modification of antibody carbohydrate domain and azide attachment

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 (page 4):

- 75 µL of dH_2O
- 12.5 µL of 20X Tris buffer, pH 7.0 (Component F)
- 25 µL of buffer additive (Component G)
- 80 µL 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 µL of 20X Tris, pH 7.0 (Component F) to 9.5 mL of dH_2O 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 × *g* for 10 minutes, ensuring that one membrane panel of the concentrator faces the center of the rotor. Discard the flow-through.

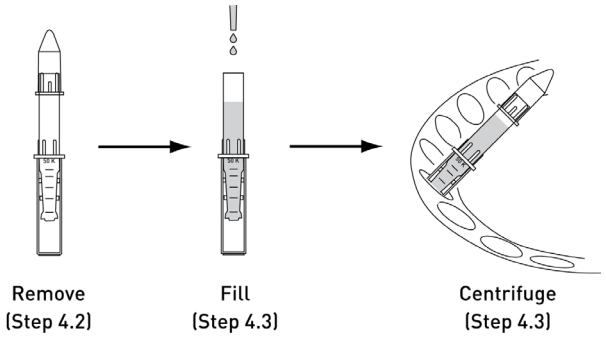

Figure 4 Wash the antibody concentrator

Purify the antibody

4.5 Add 1.6 mL of 1X Tris, pH 7.0 (or TBS) and 250 µL 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 × *g* for 6 minutes, ensuring one membrane panel of the concentrator faces the center of the rotor. Discard the flow-through.

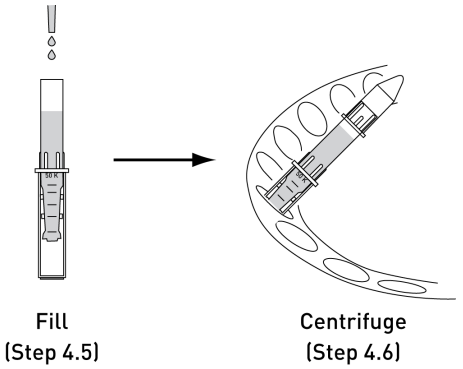

Figure 5 Purification and concentration of azide-modifed antibody

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 × *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 ~100 µL or an antibody concentration of more than ~2.0 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 antibody

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

4.10 Centrifuge at 1000 × *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 OD_{280} (with OD_{280} at 1.4 = 1 mg/mL). Expected concentration is ~1–5 mg/mL.

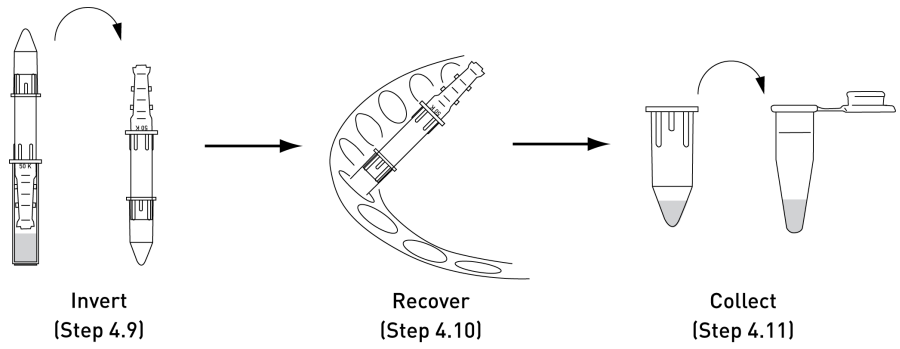

Figure 6 Collection of purified and concentrated azide-modifed antibody

Step 5. Conjugate with sDIBO-modified biotin

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

Add sDIBO-modified biotin

5.1 Add 11 µL of the Click-iT™ Biotin sDIBO alkyne (sDIBO-modified biotin, Component J) to 100 µg azide-modified antibody in 100 µL of 1X Tris pH 7.0 (or TBS) in the 1.5-mL centrifuge tube.

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 antibody conjugate", page 8) or purify it of the excess unconjugated antibody (Step 6, optional).

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

Time required: 1 hour

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

Wash the antibody concentrator

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

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 5).

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 (Component I).

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) and 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 purified antibody conjugate

6.5 Invert the antibody concentrator into the conical collection tube as shown in Figure 7.

6.6 Centrifuge at 1000 × *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.

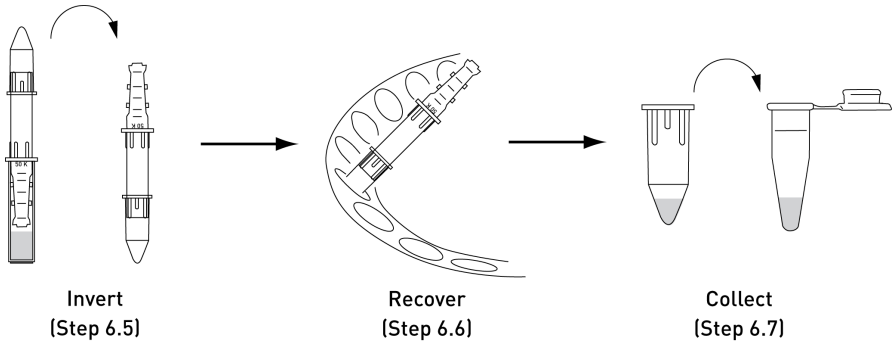

Figure 7 Collection of purified and concentrated antibody-biotin conjugate

Store the antibody conjugate

Store the antibody-biotin 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 β-Galactosidase. The azide-containing sugar, GalNAz, is then added to the modified carbohydrate domain of the antibody via the β-1,4-galactosyltransferase (GalT)-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 biotin in a copper-free click reaction with simple overnight incubation (Figure 8).

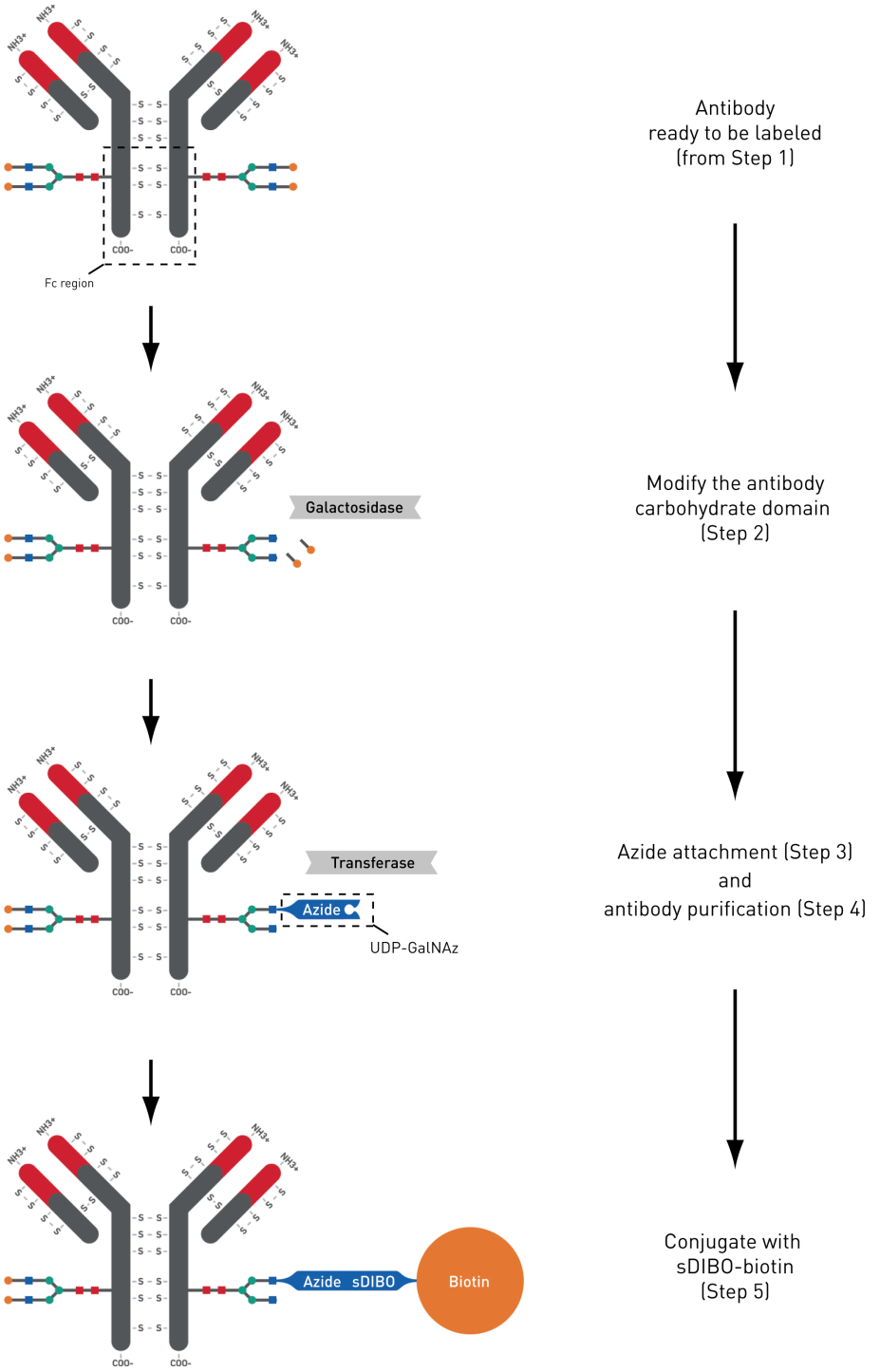

Figure 8 SiteClick™ conjugation reaction

Ordering information

Cat. No.	Product name	Unit size
S20033	SiteClick™ Biotin Antibody Labeling 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
S20026	SiteClick™ Antibody Azido Modification 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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- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**thermofisher.com/support**)
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- Download software updates and patches

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

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