

Click-iT™ sDIBO Alkyne for SiteClick™ Antibody Labeling

Catalog Nos. C20027, C20028, C20029, C20030, C20031, C20032

Pub. No. MAN0017079

Rev. B.00

Product description

The Click-iT™ sDIBO Alkyne for SiteClick™ Antibody Labeling kits allow you to create your own labeled antibody conjugate from essentially any IgG antibody produced in eukaryotic cells regardless of isotype and host species. This is achieved by covalently linking the desired Click-iT™ sDIBO Alkyne label (see Table 1) to an azide-modified antibody in a copper-free click reaction (Figure 1, page 2). You can generate the azide-modified antibody you wish to label using the SiteClick™ Antibody Azido Modification Kit (available separately, Cat. No. S20026) or use an existing antibody engineered to contain an azido moiety. When used in conjunction with the SiteClick™ Antibody Azido Modification Kit, the Click-iT™ sDIBO Alkynes give 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 Click-iT™ sDIBO Alkyne for SiteClick™ Antibody Labeling kit contains sufficient reagents to perform one conjugation reaction of a Click-iT™ sDIBO Alkyne label (see Table 1) to an azide-modified primary IgG antibody. This user guide describes a SiteClick™ conjugation reaction starting with 100–250 µg of azide-modified IgG antibody.

Table 1. Click-iT™ sDIBO Alkyne for SiteClick™ Antibody Labeling kits.

Product*	Amount	Catalog No.
Click-iT™ Alexa Fluor™ 488 sDIBO Alkyne for SiteClick™ Antibody Labeling	1 kit	C20027
Click-iT™ Alexa Fluor™ 555 sDIBO Alkyne for SiteClick™ Antibody Labeling	1 kit	C20028
Click-iT™ Alexa Fluor™ 647 sDIBO Alkyne for SiteClick™ Antibody Labeling	1 kit	C20029
Click-iT™ Biotin sDIBO Alkyne for SiteClick™ Antibody Labeling	1 kit	C20030
Click-iT™ Amine sDIBO Alkyne for SiteClick™ Antibody Labeling	1 kit	C20031
Click-iT™ SDP Ester sDIBO Alkyne for SiteClick™ Antibody Labeling	1 kit	C20032

* See Table 2 (page 2) for kit contents and storage.

Table 2. Contents and storage

Material	Amount	Storage*
Catalog Nos. C20027, C20028, C20029, C20030, C20031		
Click-iT™ sDIBO Alkyne for SiteClick™ Antibody Labeling (Component A)†	25 µL in DMSO	<ul style="list-style-type: none"> • 2–8°C • DO NOT FREEZE
Antibody concentrator (large) (Component B)	each	
Catalog No. C20032		
Click-iT™ SDP Ester sDIBO Alkyne for SiteClick™ Antibody Labeling (Component A)	55 µg, lyophilized	<ul style="list-style-type: none"> • 2–8°C • DO NOT FREEZE
Dimethylsulfoxide (DMSO) (Component B)	100 µL	
Antibody concentrator (large) (Component C)	each	
* When stored as directed, the kit is stable for at least 6 months. † See Table 1 (page 1) for the Click-iT™ sDIBO Alkyne included in the kit by catalog number.		

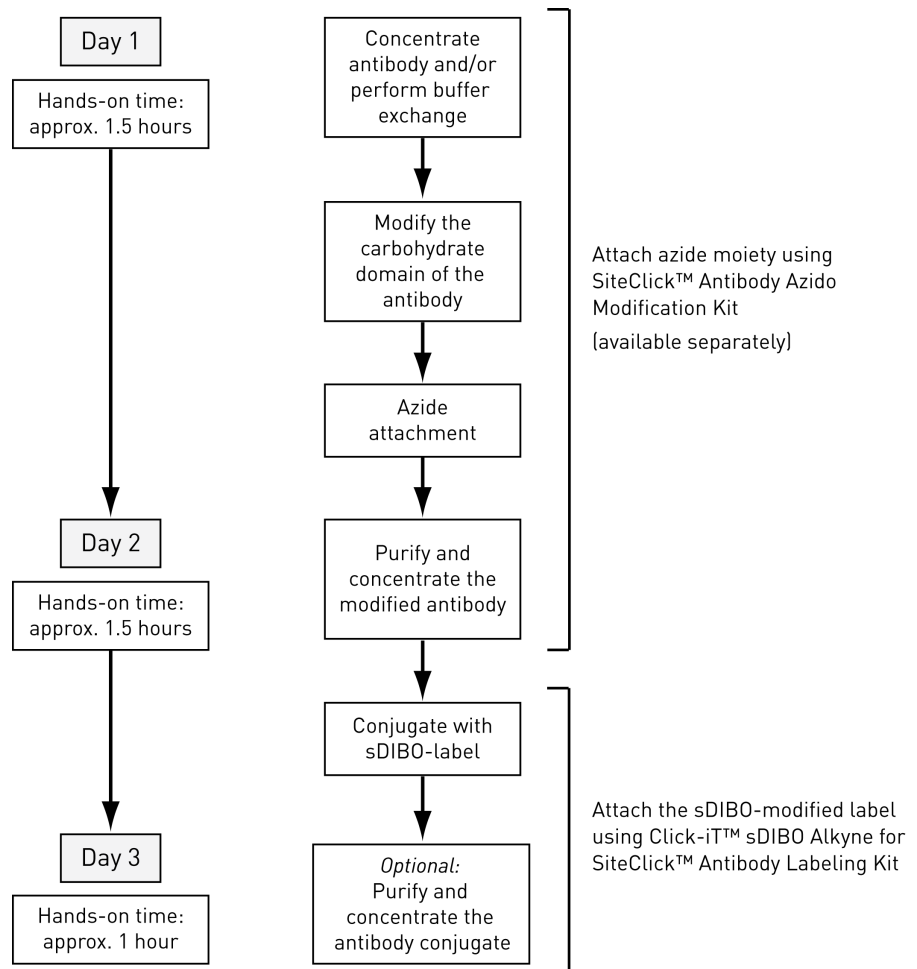


Figure 1. SiteClick™ antibody labeling workflow. The Click-iT™ sDIBO Alkyne for SiteClick™ Antibody Labeling kits are designed to be used with the SiteClick™ Antibody Azido Modification Kit (available separately; Cat. No. S20026) or with engineered antibodies containing azido moieties.

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 azide-modified IgG antibody, at a concentration of ~1 mg/mL in a Tris-based buffer, free of carrier proteins and/or azide.
 - 1.5-mL centrifuge tubes
 - Distilled water (dH₂O)
 - 1X Tris, PBS, or TBS buffer
- 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, 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.

Step 1. Attach sDIBO-modified label to azide-modified antibody

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

IMPORTANT! The following protocol requires the use of azide-modified antibodies to create your own labeled antibody conjugate. We recommend using the SiteClick™ Antibody Azido Modification Kit (Cat. No. S20026) to specifically attach an azide moiety to the heavy chains of your unlabeled IgG antibody (other engineered antibodies containing azido moieties are also suitable).

This section provides instructions to covalently attach a Click-iT™ sDIBO Alkyne label to an azide-modified antibody in a copper-free click reaction. For instructions on how to attach an azide moiety to an unlabeled antibody using the SiteClick™ Antibody Azido Modification Kit, refer to the *SiteClick™ Antibody Azido Modification Kit User Guide* (Pub. No. MAN0017078), available for download at thermofisher.com.

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

- 1.1 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.

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

- 1.2 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 5) or purify it of the excess unconjugated antibody (Step 2, optional).

Step 2. Purify and concentrate the antibody conjugate (optional)

Time required: 1 hour

- The purification step removes any excess sDIBO-label that has not been conjugated to an antibody.
- You may use TBS or PBS for the purification and collection of the modified antibody (Steps 2.2–2.7)

Wash the antibody concentrator

- 2.1 Remove the conical collection tube from the antibody concentrator (Component B; Component C in Cat. No. C20032).
- 2.2 Add 1X Tris, TBS, or PBS to a total volume of 2 mL to the antibody concentrator as shown in Figure 2.
- 2.3 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.

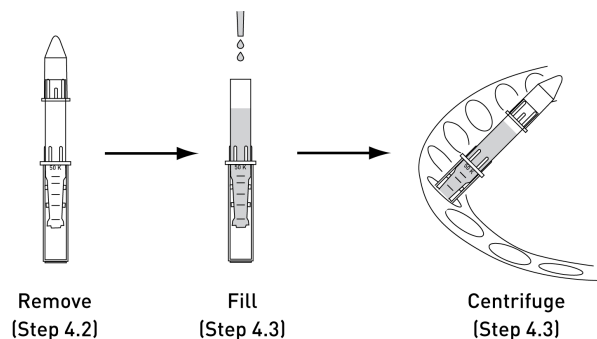


Figure 2. Wash the antibody concentrator.

Purify the antibody conjugate

- 2.4 Add 1.6 mL of 1X Tris, TBS, or PBS to the antibody concentrator, then add the sDIBO-modified antibody (from Step 1.2) to the same concentrator.
- 2.5 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.
- 2.6 Add 1.8 mL of 1X Tris, TBS, or PBS to the antibody concentrator, then centrifuge at $1200 \times g$ for 10 minutes, ensuring that one membrane panel of the concentrator faces the center of the rotor.
- 2.7 Discard the flow-through and repeat Step 2.6 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 $1200 \times g$ for an additional 5 minutes or until the appropriate volume is achieved).

Collect the purified antibody conjugate

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

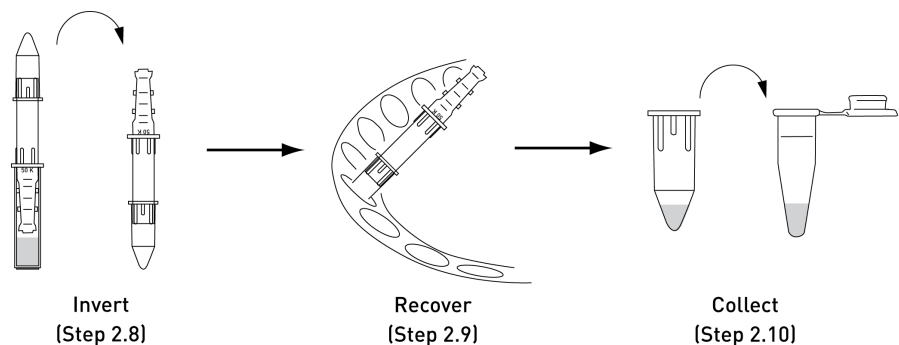


Figure 3. Optional purification and concentration of the labeled antibody conjugate.

Store the antibody conjugate

Store the antibody-biotin conjugate at $2-8^{\circ}\text{C}$ 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 (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 4).

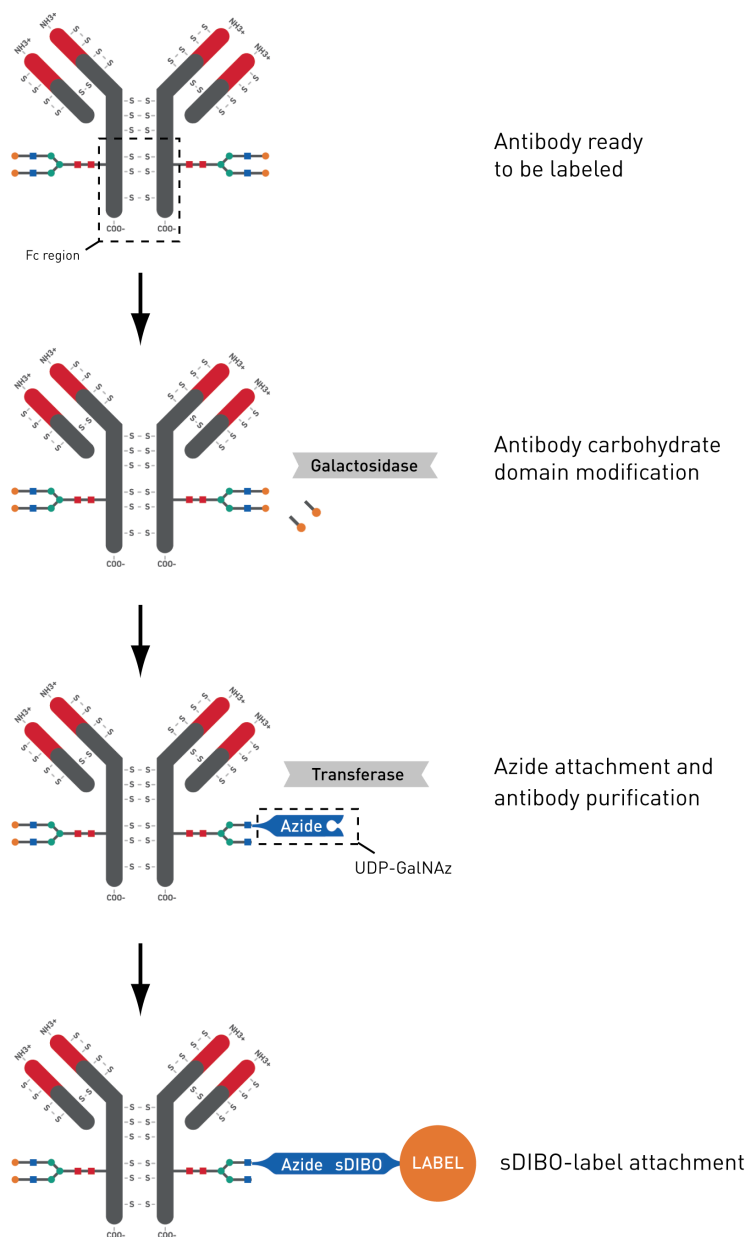


Figure 4. SiteClick™ conjugation reaction.

Ordering information

Cat. No.	Product name	Unit size
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

Related products

S20026	SiteClick™ Antibody Azido Modification Kit.	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.

Obtaining support

For the latest services and support information for all locations, go to thermofisher.com/support.

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (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

Safety Data Sheets (SDSs) are available at thermofisher.com/support.

Certificate of Analysis

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Revision history: Pub. No. MAN0017079

Revision	Date	Description
B.00	12 July 2017	Clarify that Step 2 (Purification and concentration) removes unconjugated, excess sDIBO label.
A.00	11 May 2017	New User Guide

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Manufacturer: Life Technologies Corporation | 29851 Willow Creek Road | Eugene, OR 97402

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