SiteClick™ Antibody Azido Modification Kit *1 mg labeling*

Catalog No. S10900

Pub. No. MAN0018872 **Rev.** B.0

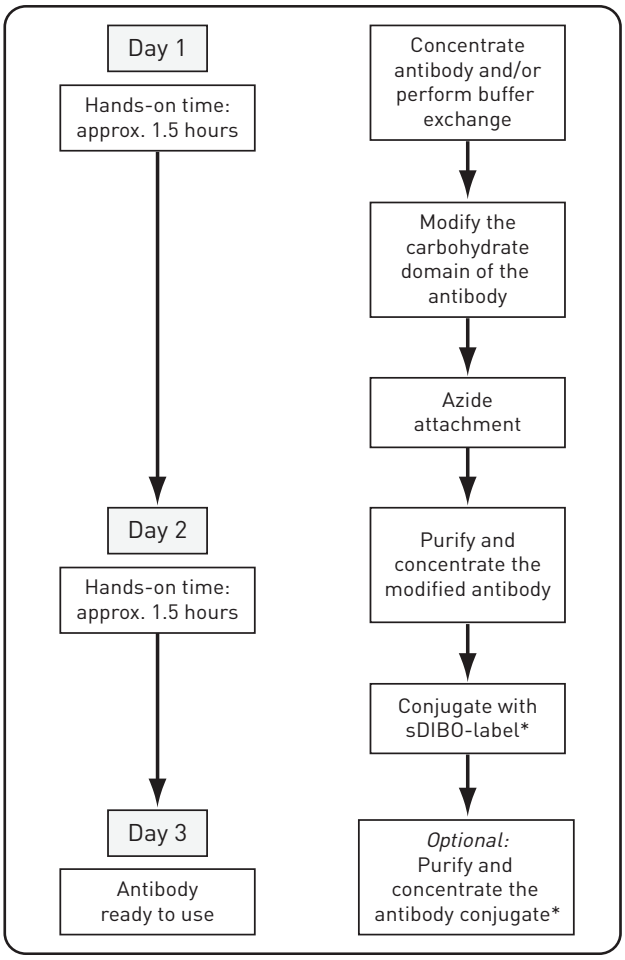
Product information

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 SiteClick™ sDIBO Alkyne labels (available separately; see Table 2, page 3) 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 1 mg of whole IgG produced in eukaryotic cells from any host species. 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	• 2–8°C • DO NOT FREEZE
Antibody concentrator (small) (Component B)	each	
Collection tube (Component C)	each	
ß-Galactosidase (Component D)	24 µL	
UDP-GalNAz (Component E)	440 µg	
20X Tris pH 7.0 (1M) (Component F)	1.8 mL	
Buffer additive (Component G)	60 µL	
ß-1,4-galactosyltransferase (GalT) (Component H)	176 µL	
Antibody concentrator (large) (Component I)	each	
* When stored as directed, this kit is stable for at least 6 months.		



* Requires the use of the SiteClick™ 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 SiteClick™ sDIBO Alkyne for SiteClick™ Antibody Labeling kits (available separately; see Table 2) for a complete 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

Required materials not supplied

- 1 mg 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
- SiteClick™ sDIBO Alkyne label (sDIBO-dye, sDIBO-biotin, or sDIBO-chelator) (Table 2, page 3).

Table 2 SiteClick™ sDIBO Alkynes for SiteClick™ Antibody Labeling. The SiteClick™ sDIBO Alkyne labels (available separately) are used in conjunction with the SiteClick™ Antibody Azido Modification Kits (sufficient for 1 mg azide-modified antibody) or with engineered antibodies containing azido moieties to create high-quality antibody conjugates.

	Catalog No.[1]		
Product	**100 µg kit**	**1 mg kit**	**5 mg kit**
SiteClick™ Biotin sDIBO Alkyne	C20030	S10902	S10907
SiteClick™ pHrodo™ iFL Red sDIBO Alkyne	C20034	S10903	S10908
SiteClick™ pHrodo™ Deep Red sDIBO Alkyne	S10914	S10915	—
SiteClick™ Alexa Fluor™ 488 sDIBO Alkyne	C20027	S10904	S10909
SiteClick™ Alexa Fluor™ 555 sDIBO Alkyne	C20028	—	—
SiteClick™ Alexa Fluor™ 647 sDIBO Alkyne	C20029	S10906	S10911

[1] See Table 3 (page 9) for the amount of SiteClick™ sDIBO Alkyne label required to label 100 µg, 1 mg, and 5 mg azide-modified antibody with the SiteClick™ sDIBO Alkyne Kits available from Thermo Fisher Scientific.

Caution

- **IMPORTANT!** Avoid sodium azide 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

Time required: 1 hour

This antibody concentration and buffer exchange step is required if:

- Your antibody concentration is less than 10 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.

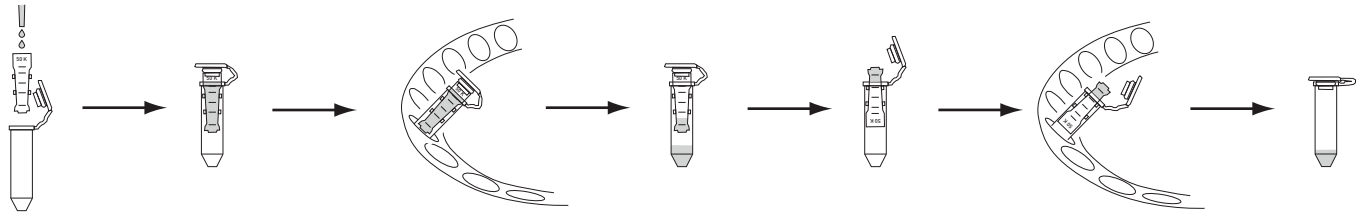


Figure 2 Antibody concentration and/or buffer exchange

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 3).

1.2 Ensure that the cap strap and one membrane panel of the concentrator faces the center of the rotor, then centrifuge at 5000 × *g* for 6 minutes.

1.3 Discard the flow-through.

Concentrate antibody and/or perform buffer exchange

1.4 Add a sufficient volume of antibody solution to contain 1 mg of antibody to the small antibody concentrator (Component B).

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

1.6 Ensure that the cap strap and one membrane panel of the concentrator faces the center of the rotor, then centrifuge at 5000 × *g* for 6 minutes.

1.7 Discard the flow-through.

1.8 Add antibody preparation buffer (Component A) to the small antibody concentrator (Component B) so that the total volume in the concentrator is 500 µL.

1.9 Ensure that the cap strap and one membrane panel of the concentrator faces the center of the rotor, then centrifuge at 5000 × *g* for 6 minutes.

Note: If the antibody volume in the concentrator is greater than 100 µL following Step 1.9, centrifuge at 5000 × *g* for an additional 3 minutes or until the appropriate volume is achieved.

1.10 Invert the small antibody concentrator (Component B) into the collection tube (Component C) as shown in Figure 2.

1.11 Centrifuge for 3 minutes at 1000 × *g* to collect the concentrated antibody. Following collection, you should have approximately 100 µL 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 ß-galactosidase

2.1 Add 20 µL of β-galactosidase (Component D) to the antibody collected in Step 1.11, 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.

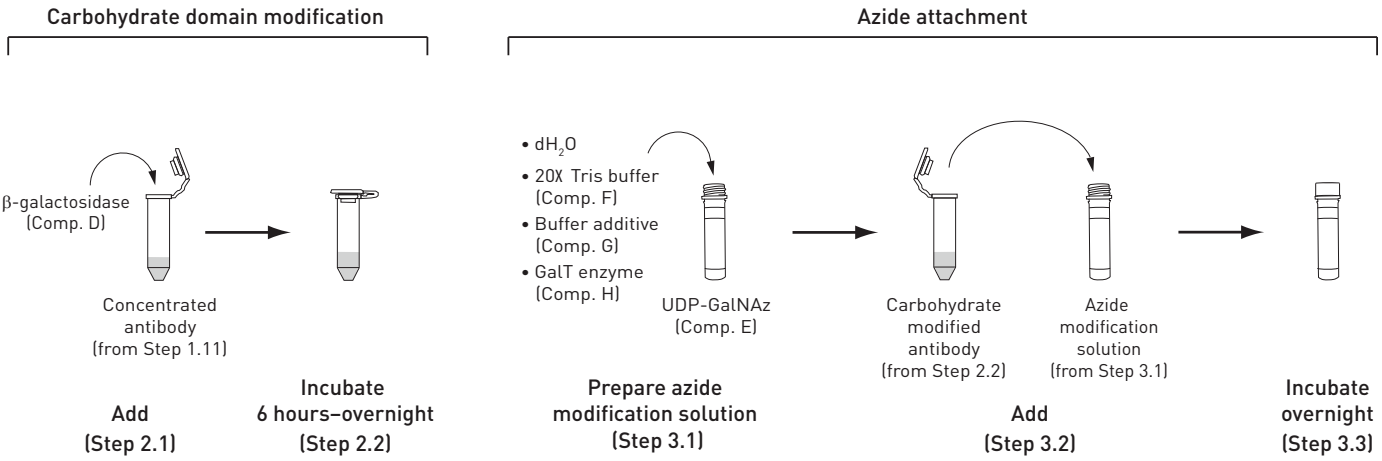


Figure 3 Modification of antibody carbohydrate domain and azide attachment

Step 3. Azide attachment

Time required: 10 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 5):

- 12 µL of dH_2O
- 18 µL of 20X Tris buffer, pH 7.0 (Component F)
- 40 µL of buffer additive (Component G)
- 160 µL of GalT enzyme (Component H)

3.2 Vortex the reaction components, then add the modified antibody from Step 2.2 (120 µL) 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: 2 hours

- This step removes any excess substrate UDP-GalNAz.
- You can 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 2 mL of 1X Tris, pH 7.0 (or TBS) to the large antibody concentrator (Component I) as shown in Figure 4 (page 6).

4.4 Ensure that one membrane panel of the concentrator faces the center of the rotor, then centrifuge at 1200 × *g* for 10 minutes. Discard the flow-through.

IMPORTANT! To avoid damage to the antibody concentrator during centrifugation, ensure that it is properly assembled and seated at the bottom of the rotor. The rim of the concentrate collection tube should be inside the rotor well. Check clearance before centrifugation.

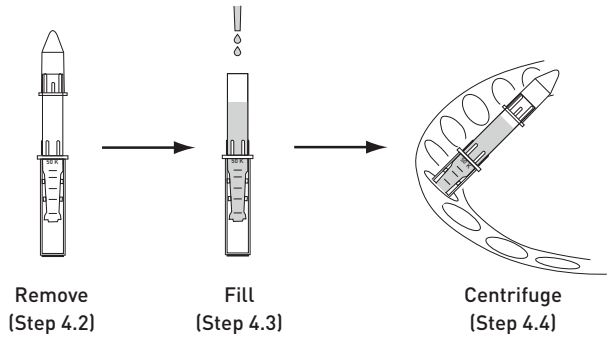


Figure 4 Wash the antibody concentrator

Purify the antibody

4.5 Add 1.5 mL of 1X Tris pH 7.0 (or TBS) and 350 µL of the azide-modified antibody from Step 3.3 to the large antibody concentrator (Component I) as shown in Figure 5.

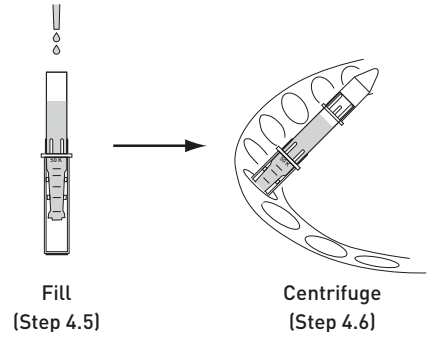


Figure 5 Purification and concentration of azide-modifed antibody

4.6 Ensure that one membrane panel of the concentrator faces the center of the rotor, then centrifuge at 1200 × *g* for 8 minutes. Discard the flow-through.

4.7 Add 1X Tris pH 7.0 (or TBS) to a total volume of 2 mL to the large antibody concentrator (Component I). Ensure that one membrane panel of the concentrator faces the center of the rotor, then centrifuge at 1200 × *g* for 11 minutes.

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 ~450 µL or if 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).

Note: If you intend to conjugate the azide-modified antibody to macromolecule-sDIBO, measure the OD_{260} in the flow-through (with ε_{260} = 9900 M^{-1} cm^{-1}) following the removal of UDP-GalNAz. In this case, an additional Step 4.7 might be necessary to remove free UDP-GalNAz sufficiently.

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 × *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 Measure the OD_{280} (with OD_{280} at 1.4 = 1 mg/mL) to determine the antibody concentration. Expected concentration is ~2–5 mg/mL.

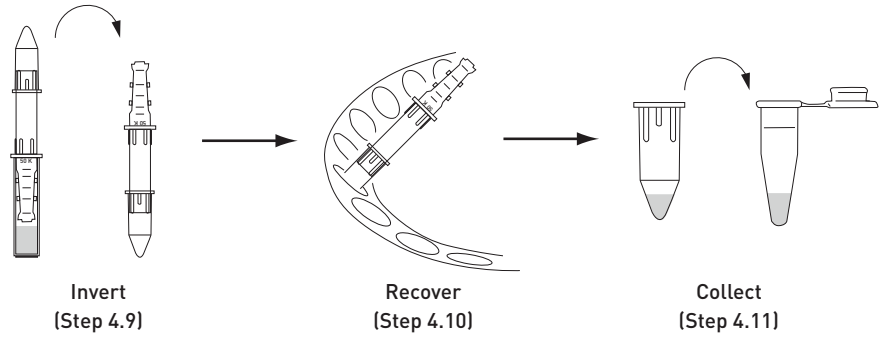


Figure 6 Collection of purified and concentrated azide-modifed antibody

Store the azide-modified antibody

At this point, you can store the azide-modified antibody at 2–8°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 SiteClick™ 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

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

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

See Table 3 (page 9) for the amount of SiteClick™ sDIBO Alkyne label required to label 100 µg, 1 mg, and 5 mg azide-modified antibody with the SiteClick™ sDIBO Alkyne Kits available from Thermo Fisher Scientific.

Materials required but not provided

- Azide-modified antibody (from Step 4.11) in a Tris-based buffer, free of carrier proteins and/or azide
- SiteClick™ sDIBO Alkyne label (available separately; see Table 3, page 9)
- Anhydrous DMSO (only required for dissolving SiteClick™ pHrodo™ iFL Red sDIBO Alkyne; included in Cat. No. S10903)
- Distilled water (dH_2O)
- 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**, 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 SiteClick™ sDIBO Alkyne to azide-modified antibody

5.1 Bring 1 mg azide-modified antibody (from Step 4.11) to a volume of 450 µL with 1X Tris pH 7.0 (or TBS) in the 1.5-mL centrifuge tube, then add 50 µL of SiteClick™ sDIBO Alkyne label.

Note: The SiteClick™ pHrodo™ iFL Red sDIBO Alkyne for SiteClick™ Antibody Labeling (Cat. No. S10903) is supplied lyophilized as a solid powder. Before use, dissolve the SiteClick™ pHrodo™ iFL Red sDIBO Alkyne in 50 µL of anhydrous DMSO, which is included in the kit.

Other SiteClick™ sDIBO Alkynes for SiteClick™ Antibody Labeling are supplied as 50-µL solutions in DMSO and do not need to be dissolved.

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 11) or purify it of the excess SiteClick™ sDIBO Alkyne label (Step 6, optional).

Table 3 SiteClick™ sDIBO Alkynes for SiteClick™ Antibody Labeling. The SiteClick™ sDIBO Alkyne labels (available separately) are used in conjunction with the SiteClick™ Antibody Azido Modification Kits or with engineered antibodies containing azido moieties to create high-quality antibody conjugates.

	Catalog No.[4]			100 µg antibody		1 mg antibody		5 mg antibody	
Product	100 µg	1 mg	5 mg	in TBS[5]	sDIBO[6]	in TBS[5]	sDIBO[6]	in TBS[5]	sDIBO[6]
SiteClick™ Biotin sDIBO Alkyne	C20030	—	—	90 µL	10 µL	—	—	—	—
SiteClick™ pHrodo™ iFL Red sDIBO Alkyne[1]	C20034	—	—	90 µL	10 µL	—	—	—	—
SiteClick™ pHrodo™ Deep Red sDIBO Alkyne	S10914	—	—	90 µL	10 µL	—	—	—	—
SiteClick™ Alexa Fluor™ 488 sDIBO Alkyne	C20027	—	—	90 µL	10 µL	—	—	—	—
SiteClick™ Alexa Fluor™ 555 sDIBO Alkyne	C20028	—	—	90 µL	10 µL	—	—	—	—
SiteClick™ Alexa Fluor™ 647 sDIBO Alkyne	C20029	—	—	90 µL	10 µL	—	—	—	—
SiteClick™ Biotin sDIBO Alkyne	—	S10902	—	45 µL	5 µL	450 µL	50 µL	—	—
SiteClick™ pHrodo™ iFL Red sDIBO Alkyne[2]	—	S10903	—	45 µL	5 µL	450 µL	50 µL	—	—
SiteClick™ pHrodo™ Deep Red sDIBO Alkyne	—	S10915	—	45 µL	5 µL	450 µL	50 µL	—	—
SiteClick™ Alexa Fluor™ 488 sDIBO Alkyne	—	S10904	—	45 µL	5 µL	450 µL	50 µL	—	—
SiteClick™ Alexa Fluor™ 647 sDIBO Alkyne	—	S10906	—	45 µL	5 µL	450 µL	50 µL	—	—
SiteClick™ Biotin sDIBO Alkyne	—	—	S10907	45 µL	5 µL	450 µL	50 µL	2.25 mL	250 µL
SiteClick™ pHrodo™ iFL Red sDIBO Alkyne[3]	—	—	S10908	45 µL	5 µL	450 µL	50 µL	2.25 mL	250 µL
SiteClick™ Alexa Fluor™ 488 sDIBO Alkyne	—	—	S10909	45 µL	5 µL	450 µL	50 µL	2.25 mL	250 µL
SiteClick™ Alexa Fluor™ 647 sDIBO Alkyne	—	—	S10911	45 µL	5 µL	450 µL	50 µL	2.25 mL	250 µL

[1–3] Dissolve SiteClick™ pHrodo™ iFL Red sDIBO Alkyne in 25 µL[1], 50 µL[2], and 250 µL[3] DMSO, respectively.
[4] 100 µg kits, 1 mg kits, and 5 mg kits contain sDIBO in 25 µL, 50 µL, and 250 µL DMSO, respectively, enough to label 2.5 × 100 µg, 10 × 100 µg, and 50 × 100 µg azide tagged antibody.
[5] Antibody can be in 50 mM Tris (pH 7.0), TBS, PBS, or other thiol-free and sodium azide-free buffer.
[6] sDIBO-derivatives are dissolved in DMSO.

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

Time required: 1 hour

Note: For SiteClick™ pHrodo™ Deep Red (Cat. Nos. S10914, S10915) purification, proceed to "Step 7. Purify and concentrate the SiteClick™ pHrodo™ Deep Red antibody conjugate", page 11.

- The purification step removes any excess SiteClick™ sDIBO alkyne label that has not been conjugated with the antibody. This removal can be achieved by size exclusion chromatography or centrifugal filtration. For convenience, centrifugal filters have been included in SiteClick™ sDIBO Alkyne for 1 mg SiteClick™ Antibody Labeling kits (Cat. Nos. S10902, S10903, S10904, and S10906).
- You can 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 SiteClick™ sDIBO Alkyne for 1 mg SiteClick™ Antibody Labeling kits)

  Note: The antibody concentrator included in the SiteClick™ sDIBO Alkyne kits (Component B; Component C in Cat. No. S10903) is identical to the large antibody concentrator (Component I) supplied with the 1 mg SiteClick™ Antibody Azido Modification Kit.
- Distilled water (dH_2O)
- 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 2 mL of 1X Tris, TBS, or PBS to the large antibody concentrator (Component B in SiteClick™ sDIBO Alkyne kits) as shown in Figure 4 (page 6).

6.3 Ensure that one membrane panel of the concentrator faces the center of the rotor, then centrifuge at 1200 × *g* for 10 minutes. Discard the flow-through.

Purify the antibody conjugate

6.4 Add 1.3 mL of 1X Tris, TBS , or PBS and 500 µL of the sDIBO-modified antibody (from Step 5.2) to the large antibody concentrator (Component B).

6.5 Ensure that one membrane panel of the concentrator faces the center of the rotor, then centrifuge at 1200 × *g* for 12 minutes. Discard the flow-through.

6.6 Add 1X Tris, TBS, or PBS to a total volume of 2 mL to the large antibody concentrator (Component B) .

6.7 Ensure that one membrane panel of the concentrator faces the center of the rotor, then centrifuge at 1400 × *g* for 12 minutes. Discard the flow-through.

6.8 Repeat Steps 6.6 and 6.7 at least three more times.

Note: If an antibody concentration of more than ~2–4 mg/mL is desired, you can reduce the volume in the concentrator by additional centrifugation (e.g., at 1400 × *g* for an additional 3 minutes or until the appropriate volume is achieved).

Collect the purified antibody conjugate

6.9 Invert the antibody concentrator into the conical collection tube as shown in Figure 7 (page 11).

6.10 Centrifuge at 1000 × *g* for 3 minutes to collect the concentrated antibody.

6.11 Transfer the purified antibody conjugate from the conical collection tube to a new 1.5-mL centrifuge tube.

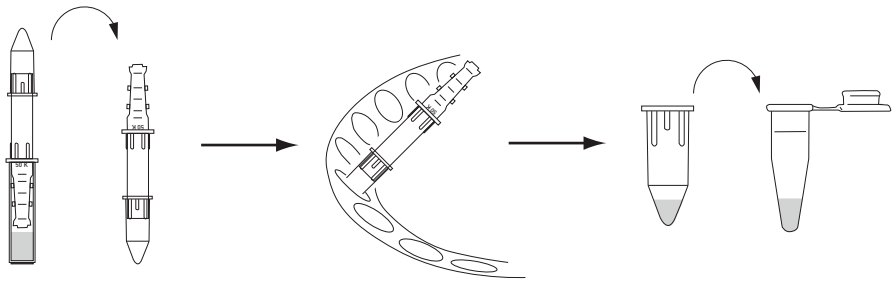


Figure 7 Optional purification and concentration of the labeled antibody conjugate

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.

Step 7. Purify and concentrate the SiteClick™ pHrodo™ Deep Red antibody conjugate *(optional)*

The following protocol describes the purification step that removes any excess SiteClick™ pHrodo™ Deep Red label that has not been conjugated with the antibody.

The pHrodo™ Deep Red dye removal column (Component C) contains a ready-to-use resin that is designed for rapid removal of pHrodo™ Deep Red dye with exceptional antibody recovery. Removal of the free dye after a labeling reaction is essential for the accurate determination of dye-to-antibody ratios. For optimal antibody recovery and dye removal, ensure that the appropriate amount of sample and buffer are used.

Materials required

- Antibody conjugate (from Step 5.2)

The following required materials are included in the SiteClick™ pHrodo™ Deep Red sDIBO Alkyne for SiteClick™ Antibody Labeling kits; Cat. No. S10914, S10915):

- pHrodo™ Deep Red dye removal column
- PBS exchange buffer
- Wash/collection vials

Procedural guidelines

- Do not reuse the purification resin
- Limit organic solvents to ≤10% of the volume

Prepare the spin column

7.1 Following the overnight reaction in step 5.2, loosen the cap on a spin column, twist the tab off of the bottom of the column, then place it into a wash vial.

Note: In Cat. No. S10915, the wash vials and collection vials are the same. Designate one as the wash vial (you can discard the lid) and the other as the collection vial (save the lid). The wash vial included in Cat. No. S10914 does not have a cap.

7.2 Centrifuge the column-tube assembly at 1,000 × *g* for 2 minutes to remove the storage buffer and pack the column.

Note: If using a fixed angle rotor, place a mark on the side of the column facing away from the rotor center. For all subsequent centrifugation steps, place the column in the microcentrifuge with the mark facing away from the rotor center.

IMPORTANT! Improper orientation of the column during centrifugation can result in reduced dye removal.

7.3 Discard the flow-through, then place the column back into the wash vial.

7.4 Add 250 µL (for Cat. No. S10914) or 500 µL (for Cat. No. S10915) of the supplied PBS exchange buffer (Component B) or the desired buffer to equilibrate the column at 1,000 × *g* for 2 minutes. Discard the flow-through.

Process the sample

7.5 Transfer the packed and equilibrated column into a fresh collection vial.

Note: In Cat. No. S10915, the collection vials have a caps.

7.6 Carefully drip the entire reaction mixture onto the column.

7.7 Centrifuge the column tube assembly at 1,000 × *g* for 2 minutes to collect the sample. Discard the column.

7.8 Note the volume collected. The antibody-pHrodo™ Deep Red conjugate is in the collection tube.

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.

Step 8. Determine the Degree of Labeling (DOL) of sDIBO alkyne-labeled antibody *(optional)*

8.1 Determine the DOL from the A_{dye}/A_{280} ratio. Use Correction Factor (CF_{280}) of the label at A_{280} to calculate (see Table 4).

$$(Moles/L)_{dye} = A_{dye}/\varepsilon_{dye}$$

$$(Moles/L)_{IgG} = [A_{280} - (CF_{280} \times A_{dye})]/203{,}000$$

$$DOL = (Moles)_{dye}/(Moles)_{IgG}$$

Table 4 Molecular weight (MW), absorption maxima (λ_{max}), molar extinction coefficient (ε_{dye}), and Correction Factor (CF_{280}) for the SiteClick™ sDIBO Alkynes for SiteClick™ Antibody Labeling.

Product	~MW	λ_{max}	ε_{dye} [1]	CF_{280} [2]
SiteClick™ pHrodo™ iFL Red sDIBO Alkyne	~1800	560 nm	65,000	0.221
SiteClick™ pHrodo™ Deep Red sDIBO Alkyne[3]	~1900	640 nm	140,000	0.33
SiteClick™ Alexa Fluor™ 488 sDIBO Alkyne	~1450	495 nm	73,000	0.134
SiteClick™ Alexa Fluor™ 555 sDIBO Alkyne	~1850	555 nm	145,000	0.091
SiteClick™ Alexa Fluor™ 647 sDIBO Alkyne	~1900	655 nm	234,000	0.037

[1] Extinction coefficient at λ_{max} in $M^{-1}cm^{-1}$.
[2] Correction factor for absorption readings (A_{280}) at 280 nm; e.g. $A_{280,actual} = A_{280,observed} - (CF_{280} \times A_{dye})$.
[3] We recommend measuring pHrodo™ Deep Red conjugates in 4.3 (wt %) phosphoric acid to obtain consistent absorbance measurements.

Example calculation with SiteClick™ Alexa Fluor™ 488 sDIBO Alkyne

Determine the DOS from the A_{495}/A_{280} ratio, using $CF_{280} = 0.134$ for Alexa Fluor™ 488 at A_{280} (from Table 4) for the calculation:

$$(Moles/L)_{dye} = A_{495}/73{,}000$$

$$(Moles/L)_{IgG} = [A_{280} - (0.134 \times A_{495})]/203{,}000$$

$$DOL = (Moles)_{dye}/(Moles)_{IgG}$$

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 8).

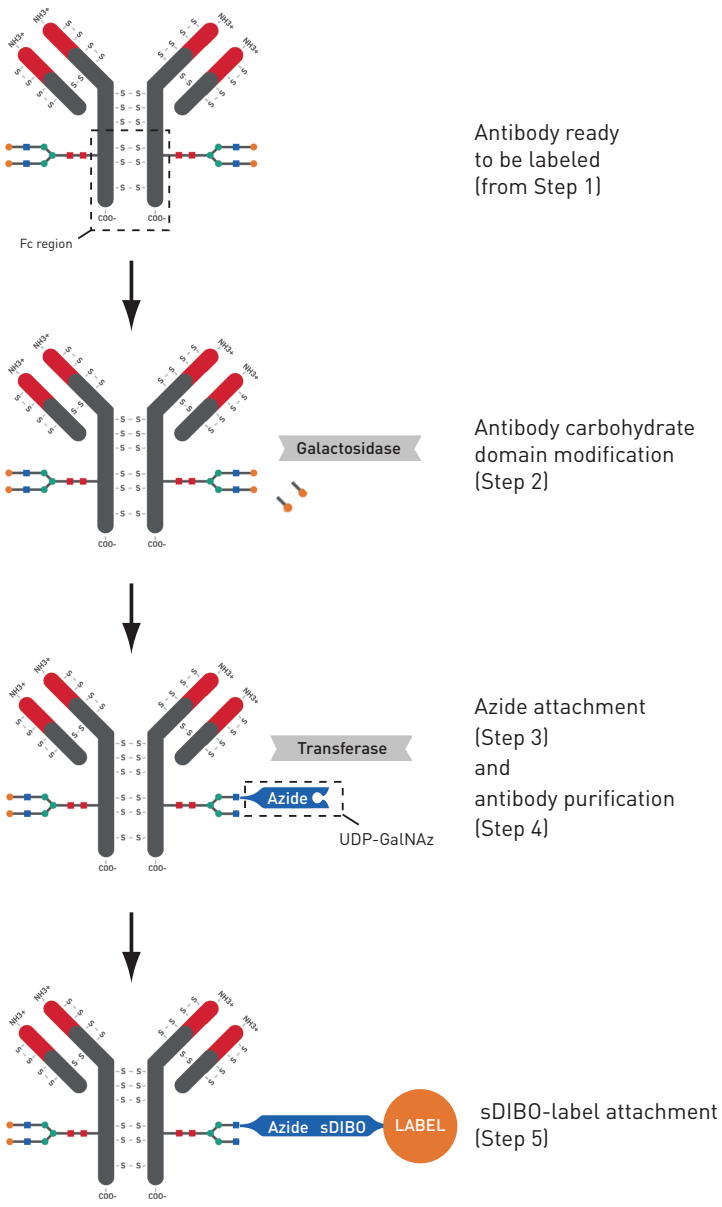


Figure 8 SiteClick™ conjugation reaction

Ordering information

Cat. No.	Product	Unit size
S10900	SiteClick™ Antibody Azido Modification Kit *1 mg labeling*	1 kit

Related products

1 mg SiteClick™ sDIBO labels for azido-modified antibodies:

S10902	SiteClick™ Biotin sDIBO Alkyne for SiteClick™ Antibody Labeling	1 kit
S10903	SiteClick™ pHrodo™ iFL Red sDIBO Alkyne for SiteClick™ Antibody Labeling	1 kit
S10904	SiteClick™ Alexa Fluor™ 488 sDIBO Alkyne for SiteClick™ Antibody Labeling	1 kit
S10906	SiteClick™ Alexa Fluor™ 647 sDIBO Alkyne for SiteClick™ Antibody Labeling	1 kit
S10915	SiteClick™ pHrodo™ Deep Red sDIBO Alkyne for SiteClick™ Antibody Labeling	1 kit

100 µg SiteClick™ sDIBO labels for azido-modified antibodies:

C20027	SiteClick™ Alexa Fluor™ 488 sDIBO Alkyne for SiteClick™ Antibody Labeling	1 kit
C20028	SiteClick™ Alexa Fluor™ 555 sDIBO Alkyne for SiteClick™ Antibody Labeling	1 kit
C20029	SiteClick™ Alexa Fluor™ 647 sDIBO Alkyne for SiteClick™ Antibody Labeling	1 kit
C20030	SiteClick™ Biotin sDIBO Alkyne for SiteClick™ Antibody Labeling	1 kit
C20031	SiteClick™ Amine sDIBO Alkyne for SiteClick™ Antibody Labeling	1 kit
C20032	SiteClick™ SDP Ester sDIBO Alkyne for SiteClick™ Antibody Labeling	1 kit
C20034	SiteClick™ pHrodo™ iFL Red sDIBO Alkyne for SiteClick™ Antibody Labeling	1 kit
S10914	SiteClick™ pHrodo™ Deep Red sDIBO Alkyne for SiteClick™ Antibody Labeling	1 kit
S20033	SiteClick™ Biotin Antibody Labeling Kit	1 kit
S20026	SiteClick™ Antibody Azido Modification Kit	1 kit

Customer and technical support

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  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited Product Warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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Revision history: Pub. No. MAN0018872

Revision	Date	Description
B.0	07 October 2020	Add protocol for SiteClick™ pHrodo™ Deep Red purification.
A.0	11 September 2019	New User Guide

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