SiteClick™ Antibody Labeling Kits

Catalog Number S10449, S10450, S10451, S10452, S10453, S10454, S10455, S10467, S10469

Pub. No. MAN0007752 Rev. A.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The SiteClick™ Antibody Labeling Kits allow you to conjugate your own antibodies to DIBO-modified Qdot™ nanocrystals (525, 565, 585, 605, 625, 655, 705, and 800-nm emission) or DIBO-modified R-Phycoerythrin (R-PE). The SiteClick™ conjugation workflow consists of three steps (antibody carbohydrate domain modification, azide attachment to the antibody, and conjugation with the DIBO-modified label) and relies on copper-free click chemistry to covalently link the label containing the DIBO moiety with the azide-modified antibody without reducing the protein. The antibody concentrators provided in the kits are used to purify and concentrate the antibody at each step of the SiteClick™ antibody labeling workflow (Figure 1 on page 3).

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 azidecontaining 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 DIBO-modified label (Qdot™ nanocrystals or R-PE) in a copper-free click reaction with simple overnight incubation (Figure 2 on page 4).

Each SiteClick™ Antibody Labeling Kit contains sufficient reagents to perform one conjugation reaction of Qdot™ nanocrystals or R-PE to a primary IgG antibody sample. The protocol in this manual describes a conjugation reaction starting with 100–125 µg of whole IgG from any host species.

Table 1 Optimal fluorescence excitation and emission maxima of DIBO-modified labels

Label	Excitation (nm)[1]	Emission (nm)[1]	Extinction coefficient (ε) (M^{-1} cm^{-1})	Measured at (nm)	Cat. No.
Qdot™ 525	<525 nm	525 nm	200,000 M^{-1} cm^{-1}	between 504 nm and 512 nm	S10449
Qdot™ 565	<565 nm	565 nm	300,000 M^{-1} cm^{-1}	between 548 nm and 556 nm	S10450
Qdot™ 585	<585 nm	585 nm	250,000 M^{-1} cm^{-1}	between 572 nm and 580 nm	S10451
Qdot™ 605	<605 nm	605 nm	400,000 M^{-1} cm^{-1}	between 592 nm and 600 nm	S10469
Qdot™ 625	<625 nm	625 nm	500,000 M^{-1} cm^{-1}	between 605 nm and 612 nm	S10452
Qdot™ 655	<655 nm	655 nm	1,700,000 M^{-1} cm^{-1}	550 nm	S10453
Qdot™ 705	<705 nm	705 nm	1,700,000 M^{-1} cm^{-1}	550 nm	S10454
Qdot™ 800	<800 nm	800 nm	1,700,000 M^{-1} cm^{-1}	550 nm	S10455
R-Phycoerythrin	496, 546, 565 nm[2]	578 nm	1,960,000 M^{-1} cm^{-1}	578 nm	S10467

[1] Qdot™ nanocrystals are excitable (Ex, in nm) at any wavelength below their emission maxima (Em, in nm). For most practical applications, they can be excited at wavelengths below 405 nm.

[2] Multiple absorbance peaks

Contents and storage

Table 2 SiteClick™ Antibody Labeling Kit contents (Cat. Nos. S10449, S10450, S10451, S10452, S10453, S10454, S10455, S10467, S10469)

Component	Cap color	Amount	Storage[1]
Antibody preparation buffer (Component A)	Yellow	1.8 mL	2–8°C Do not freeze, and protect from light.
Antibody concentrator (small) (Component B)	N/A[2]	each	
Collection tube (Component C)	N/A	each	
β-Galactosidase (Component D)	Green	12 µL	
UDP-GalNAz (Component E)	Blue	220 µg	
20X Tris pH 7.0 (Component F)	Red	1.8 mL	
Buffer additive (Component G)	Purple	30 µL	
β-1,4-galactosyltransferase (GalT) (Component H)	Orange	88 µL	
Antibody concentrator (large) (Component I)	N/A	each	
DIBO-modified label (Component J)[3]	Dark Orange	55 µL (Qdot™) or 80 µL (R-PE)	
Purification concentrator (Component K)[4]	N/A	each	

[1] When stored as directed, this kit is stable for at least 3 months.
[2] N/A = not applicable.
[3] DIBO-modified Qdot™ nanocrystal or DIBO-modified R-Phycoerythrin (R-PE). See Table 1 for the approximate fluorescence excitation and emission maxima.
[4] Only available with SiteClick™ Qdot™ 605 Antibody Labeling Kit (Cat. No. S10469).

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier. Catalog numbers that appear as links open the web pages for those products.

Item	Source
Equipment	
Centrifuge with fixed angle rotor that can accommodate 1.5-mL centrifuge tubes	MLS
Centrifuge with swinging bucket or fixed angle rotor with 17 mm × 100 mm inserts	MLS
Reagents and consumables	
100 to 125 µg of whole IgG antibody[1]	MLS
Distilled water (dH_2O)	MLS
Centrifuge tubes: 1.5-mL and 15-mL	MLS

[1] Preferably at a concentration of 2 to 4 mg/mL in a Tris-based buffer, free of carrier proteins and/or azide.

Workflow

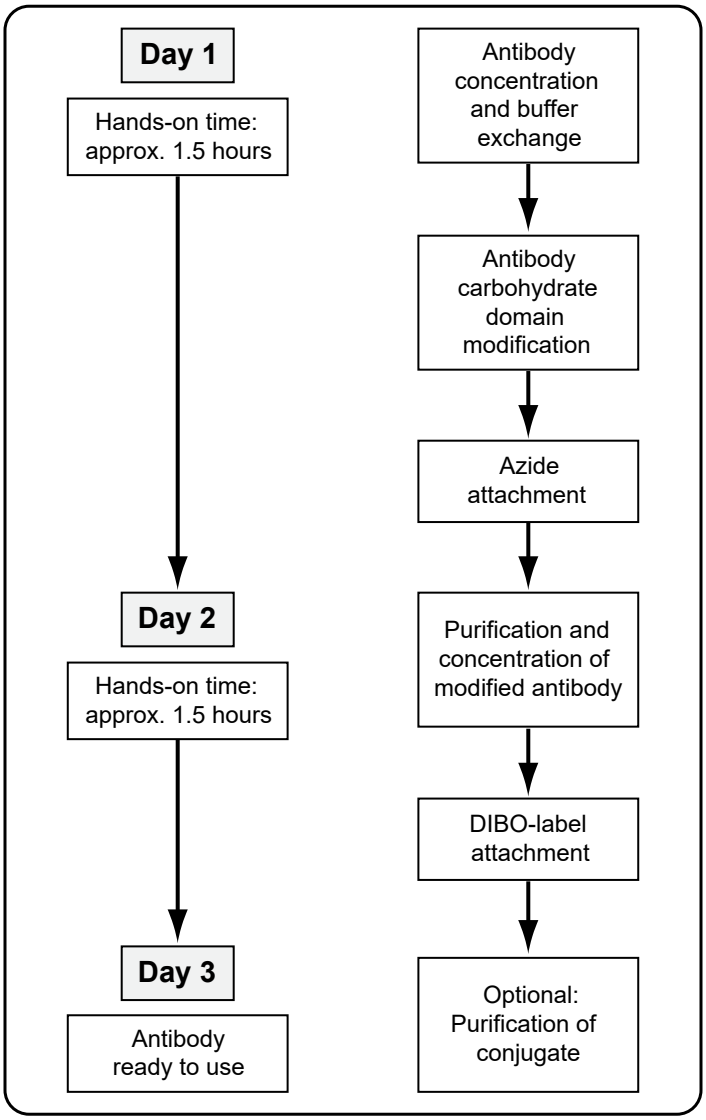

Figure 1 SiteClick™ antibody labeling workflow

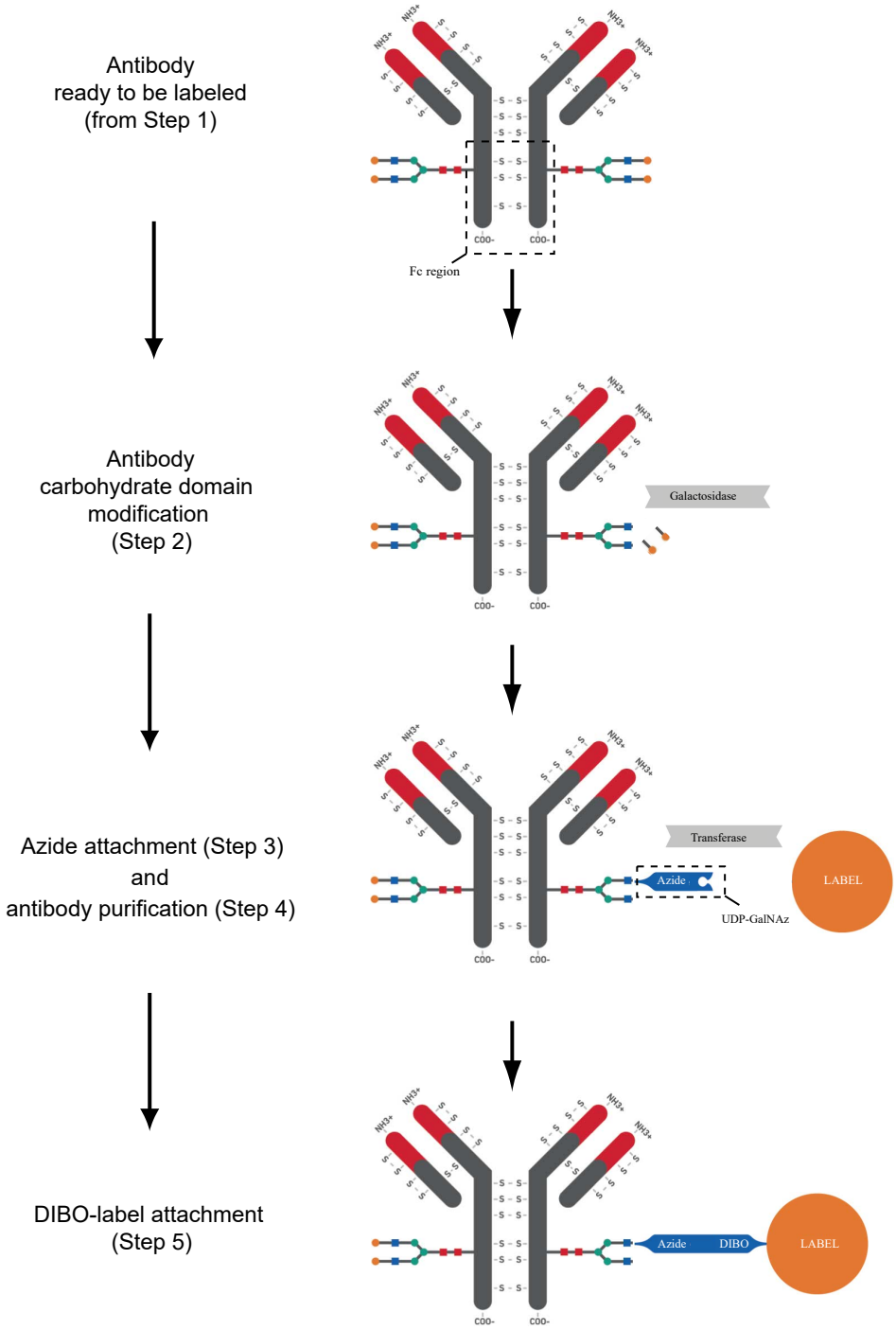

Figure 2 SiteClick™ conjugation reaction

Caution

- β-Galactosidase (Component D) can cause an allergic skin reaction, and it can cause allergy or asthma symptoms or breathing difficulties, if inhaled.
- The Qdot™ conjugate contains cadmium and selenium in an inorganic crystalline form.
- Discard the reagents in compliance with all pertaining local regulations.
- If there is contact with the eyes, rinse the eyes immediately with plenty of water and seek medical advice.
- Always wear appropriate laboratory protective clothing and gloves when handling these reagents.

Step 1. Antibody concentration and/or Buffer exchange *(Optional)*

Time required: 1 hour

Perform the antibody concentration and buffer exchange step if:

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

Before you start, briefly centrifuge Components A, C, D, E, F, G, H, and J to ensure that all material is at the bottom of the tubes.

Wash the antibody concentrator

1. Add 450 µL of dH_2O to the small antibody concentrator (Component B), then cap the device (see Figure 3).
2. Centrifuge for 6 minutes at 5000 × *g*.

   Note: Ensure that the cap strap and one membrane panel of the concentrator faces the center of the rotor.
3. Discard the flow-through.

Concentrate the antibody and the exchange buffer

1. Add a sufficient volume of antibody solution to contain 100–125 µg of antibody to the small antibody concentrator. For example, if the antibody concentration is 1 mg/mL, add 125 µL.
2. Dilute the added antibody to 500 µL using the antibody preparation buffer (Component A).
3. Centrifuge for 6 minutes at 5000 × *g*. Ensure that the cap strap and one membrane panel of the concentrator faces the center of the rotor.
4. Discard the flow-through.
5. Add 450 µL of antibody preparation buffer (Component A) to the small antibody concentrator (Component B), then centrifuge for 6 minutes at 5000 × *g*. Ensure 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 after Step 5, centrifuge for an extra 3 minutes at 5000 × *g* or until the appropriate volume is achieved.
6. Invert the small antibody concentrator (Component B) into the collection tube (Component C) (see Figure 3).
7. Centrifuge for 3 minutes at 1000 × *g* to collect the concentrated antibody. After collection, you should have approximately 50 µL of concentrated antibody in the collection tube.

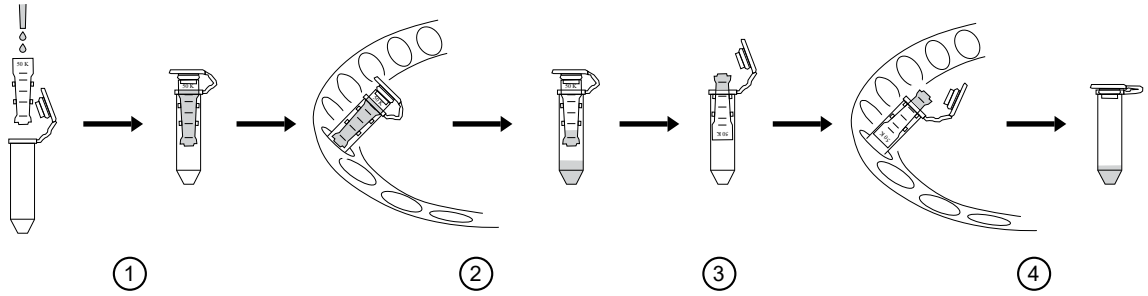

Figure 3 Antibody concentration and/or buffer exchange

① Add antibody and antibody preparation buffer to antibody concentrator

② Centrifuge

③ Invert the antibody concentrator into collection tube

④ Centrifuge to collect the concentrated antibody

Step 2. Modification of the antibody carbohydrate domain

Time required: 4 hours, hands-off

Add β-galactosidase

1. Add 10 µL of β-galactosidase (Component D) to the antibody collected in step 7 on page 5 (see Figure 4).
2. Wrap the tube cap with Parafilm™ laboratory film or similar, then incubate for 4 hours at 37°C.

Step 3. Azide attachment

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

Add GalT enzyme

1. Prepare the azide modification solution by adding the following components to the tube containing UDP-GalNAz (Component E) (see Figure 4):
   - 75 µL of dH_2O
   - 10 µL of 20X Tris buffer, pH 7.0 (Component F)
   - 25 µL of buffer additive (Component G)
   - 80 µL of GalT enzyme (Component H)
2. Vortex the reaction components, then add the modified antibody (from "Add β-galactosidase", step 2 on page 6) to the tube.
3. Briefly centrifuge the tube, wrap the tube cap with Parafilm™ laboratory film or similar, then incubate overnight at 30°C.

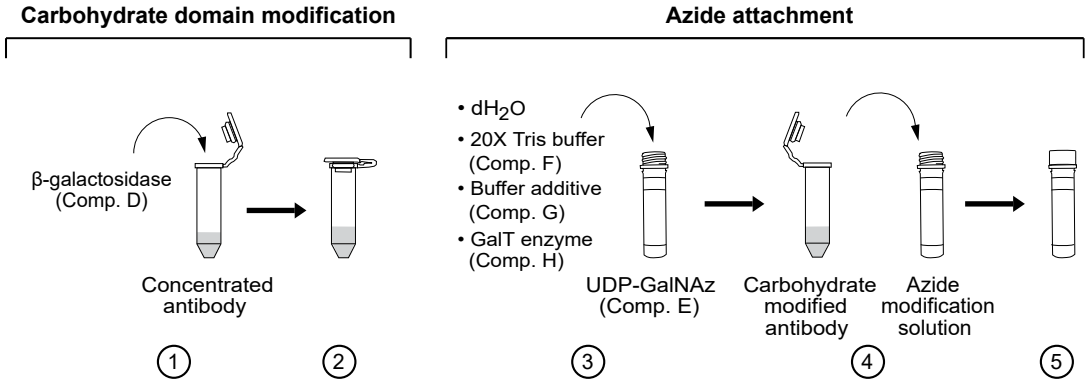

Figure 4 Modification of antibody carbohydrate domain and azide attachment

① Add β-galactosidase to the concentrated antibody
② Incubate for 4 hours
③ Prepare the azide modification solution
④ Add the azide modification solution to the modified antibody
⑤ Incubate overnight

Step 4. Purification and concentration of the azide-modified antibody

Time required: 1 hour

Wash the antibody concentrator

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.

   Note: You can also use TBS for the purification and collection of the modified antibody. 20X Tris, pH 7.0 is provided for convenience.
2. Remove the conical collection tube from the large antibody concentrator (Component I) (see Figure 5).
3. Add 1 mL of 1X Tris, pH 7.0 to the large antibody concentrator (Component I), then centrifuge for 10 minutes at 1200 × *g*. Ensure that one membrane panel of the concentrator faces the center of the rotor.
4. Discard the flow-through

Purify the azide-modified antibody

1. Add 1.75 mL of 1X Tris, pH 7.0 and 250 µL of the azide-modified antibody (from "Add GalT enzyme", step 3 on page 6) to the large antibody concentrator (Component I) (see Figure 5).
2. Centrifuge for 6 minutes at 1200 × *g*. Ensure that one membrane panel of the concentrator faces the center of the rotor.
3. Discard the flow-through.
4. Add 1.8 mL of 1X Tris, pH 7.0 to the large antibody concentrator (Component I), then centrifuge for 10 minutes at 1200 × *g*. Ensure that one membrane panel of the concentrator faces the center of the rotor.
5. Discard the flow-through, then repeat step 4.

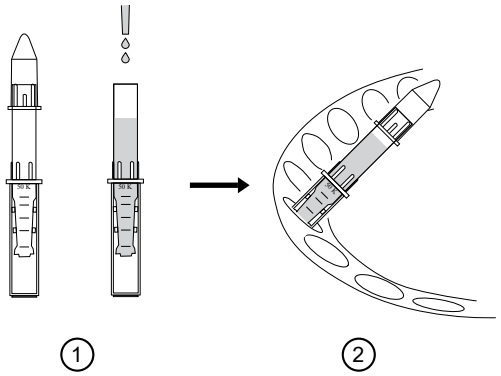

Figure 5 Purification and concentration of azide-modifed antibody

① Add azide-modified antibody to the large antibody concentrator ② Centrifuge

Concentrate and collect the purified azide-modified antibody

1. Add 1.8 mL of 1X Tris, pH 7.0 to the large antibody concentrator (Component I), then centrifuge for 10 minutes at 1400 × *g*. Discard the flow-through. The final volume in the concentrator should be approximately 80–120 µL.

   Note: If the antibody volume in the concentrator is greater than 100 µL, centrifuge for an extra 5 minutes at 1400 × *g* or until the appropriate volume is achieved.
2. Invert the antibody concentrator into the conical collection tube (see Figure 6).
3. Centrifuge for 3 minutes at 1000 × *g* to collect the concentrated antibody.
4. Transfer the antibody from the conical collection tube to a 1.5-mL centrifuge tube. If the final collected volume is less than 100 µL, dilute the antibody to 100 µL with 1X Tris, pH 7.0.

   Note: At this stage, you can store the antibody at 2–8°C and attach the DIBO-modified label at a later time.

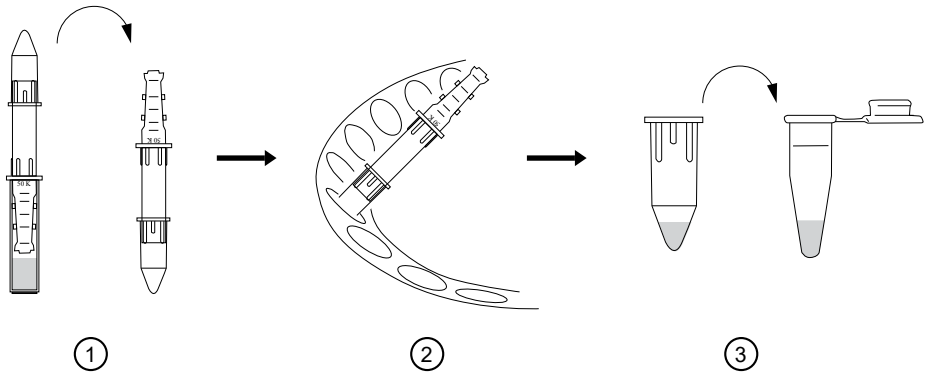

Figure 6 Collection of the purified and concentrated azide-modifed antibody

① Invert the antibody concentrator
② Centrifuge
③ Collect the concentrated antibody

Step 5. Conjugation with DIBO-modified label

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

Add DIBO-modified label

1. Add the DIBO-modified label (Component J) to the azide-modified antibody in the 1.5-mL centrifuge tube:
   - If using DIBO-modified Qdot™ nanocrystal, add 50 µL of Qdot™ DIBO (Component J).
   - If using DIBO-modified R-PE, add 75 µL of R-PE DIBO (Component J).
2. Vortex the reaction mixture, briefly centrifuge, then incubate overnight at 25°C.
3. Store the antibody conjugate at 2–8°C, protected from light (see "Antibody conjugate storage" on page 9).

   Note: *(Optional)* If desired, you can further purify the antibody conjugate of excess unconjugated antibody ("Step 6. Purification and concentration of the antibody conjugate (optional)" on page 8).

Step 6. Purification and concentration of the antibody conjugate *(optional)*

Time required: 1 hour

The purification step removes any excess antibody that has not been conjugated with the Qdot™ DIBO-modified label as well as uncojugated Qdot™ nanocrystals. Typical yield from this step is approximately 80%.

Note: The flow-through from the final concentration and purification step can be colored, and this color can vary. This is normal and does not affect the quality of the final purified conjugate.

Note: For many applications, purification of the antibody conjugate is not necessary, because the efficiency of the SiteClick™ conjugation reaction yields a minimal quantity of free antibody.

Note: The purification concentrator (Component K) is only available in SiteClick™ antibody purification kits containing DIBO-modified Qdot™ nanocrystals with emission maxima of 605, 625, 655, 705, and 800 nm. Antibody conjugates labeled with R-PE or with Qdot™ nanocrystals that emit at 525, 565, or 585 nm are too small to be retained by the membrane in the purification concentrator.

Purify and concentrate the antibody conjugate

1. Add 500 µL of dH_2O to the purification concentrator (Component K), then centrifuge for 5 minutes at 1500 × *g*. Discard the flow-through.
2. Add 150 µL of the labeled antibody conjugate (from step 2 on page 8) to the purification concentrator (Component K) (see Figure 7).
3. Add buffer (TBS or PBS) to the purification concentrator to bring the volume to 500 µL, then centrifuge for 10 minutes at 1500 × *g*. Discard the flow-through.
4. Add 500 µL of buffer (TBS or PBS) to the purification concentrator, then centrifuge for 10 minutes at 1500 × *g*.
5. Discard the flow-through, then repeat step 4.
6. Collect the purified labeled antibody conjugate from the top of the concentrator, then transfer to a new 1.5-mL centrifuge tube. You can store the conjugated antibody at 2–8°C, protected from light (see "Antibody conjugate storage" on page 9).

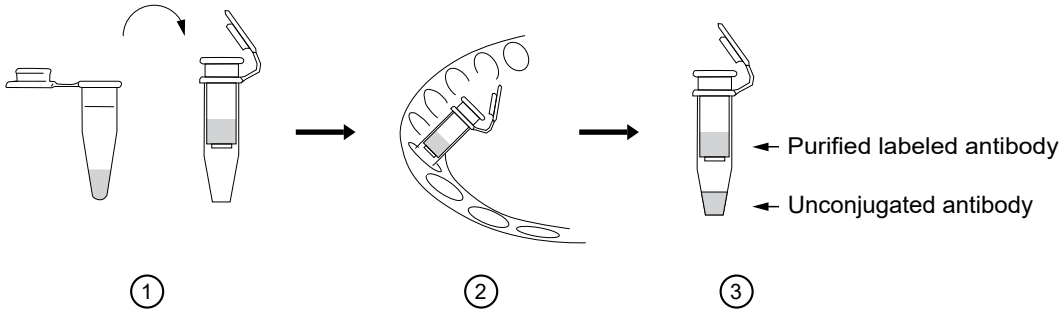

Figure 7 Optional purification of the conjugated antibody

① Add the labeled antibody conjugate to purification concentrator
② Centrifuge
③ Collect the purified labeled antibody conjugate

Storage and use of the antibody conjugates

Antibody conjugate storage

Store the antibody conjugate labeled with the Qdot™ nanocrystal or R-PE at 2–8°C, in the dark, 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.

Antibody conjugate use

You can determine the concentration of the label in the final preparation by measuring the optical density of the label at the specified wavelength, then using the formula A = εcL, where A is the absorbance, ε is the molar extinction coefficient (Table 1 on page 1), c is the molar concentration, and L is the pathlength.

For example, for the Qdot™ 605 nanocrystal, if the material eluting from the final column has A = 0.80 measured at peak between 592 nm and 600 nm in a cuvette with 1 cm pathlength, then c = A/ε = 0.80/400,000 = 2 µM of label, based on nanocrystal absorbance.

Determine the optimal concentration of any labeled antibody empirically for a particular application or experiment. Determine the optimal working concentrations by performing a titration series for the application of interest.

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN007752 A.0

Revision	Date	Description
A.0	20 June 2023	Correct the concentration of Tris buffer needed to dilute the antibody in the concentration step, and reformat the user guide. This version of the user guide (Rev. A.0) replaces the Rev. 2.0.

The information in this guide is subject to change without notice.

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