molecular probes®
by life technologies™

# SiteClick™ Antibody Labeling Kits

**Table 1** Contents and storage

| Material | Cap color | Amount | Storage* |
|---|---|---|---|
| Antibody preparation buffer (Component A) | Yellow | 1.8 mL | • 2–8°C<br>• DO NOT FREEZE<br>• Protect from light. |
| Antibody concentrator (small) (Component B) | N/A | each | |
| Collection tube (Component C) | N/A | each | |
| ß-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 | |
| ß-1,4-galactosyltransferase (GalT) (Component H) | Orange | 88 µL | |
| Antibody concentrator (large) (Component I) | N/A | each | |
| DIBO-modified label (Component J)† | Dark Orange | 55 µL (Qdot®)<br>or<br>80 µL (R-PE) | |
| Purification concentrator (Component K)‡ | N/A | each | |

* When stored as directed, this kit is stable for at least 3 months. † DIBO-modified Qdot® nanocrystal or DIBO-modified R-Phycoerythrin (R-PE). ‡ Only available with Cat. no. S10469. N/A = not applicable.

**Approximate fluorescence excitation and emission maxima:** See Table 2, page 4.

## Introduction

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, page 2).

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 DIBO-modified label (Qdot® nanocrystals or R-PE) in a copper-free click reaction with simple overnight incubation (Figure 2, below).

Each SiteClick™ Antibody Conjugation 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.

**Figure 1** SiteClick™ antibody labeling workflow

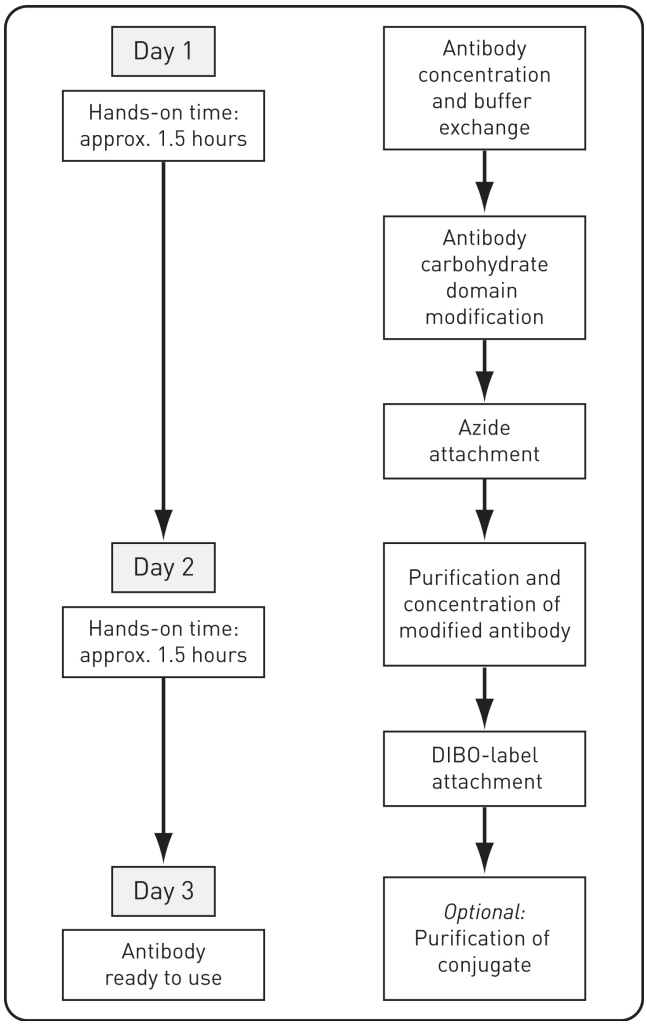
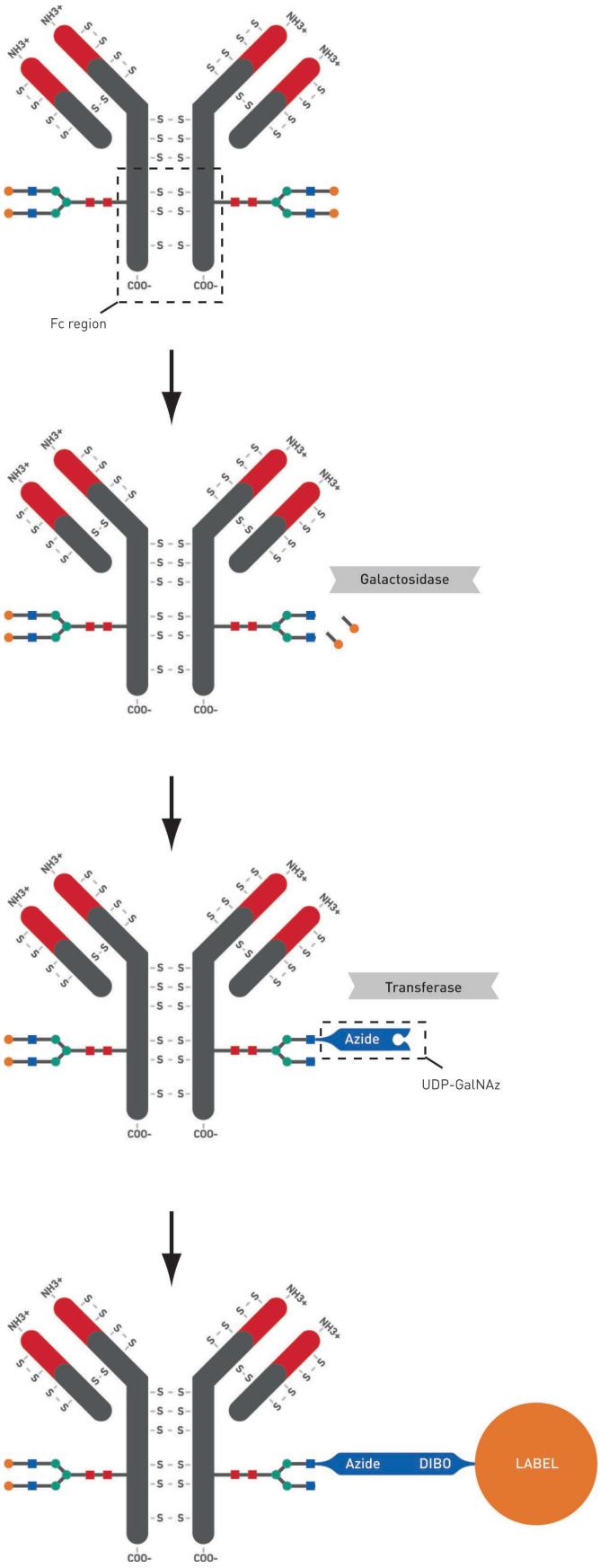


**Figure 2** SiteClick™ conjugation reaction




Antibody
ready to be labeled
(from Step 1)

Antibody
carbohydrate domain
modification
(Step 2)

Azide attachment (Step 3)
and
antibody purification (Step 4)

DIBO-label attachment
(Step 5)

**Table 2** Optimal fluorescence excitation and emission maxima of DIBO-modified labels

| Label | Ex * | Em * | Extinction coefficient (ε) | Measured At | Cat. no. |
|---|---|---|---|---|---|
| Qdot® 525 | <525 nm | 525 nm | 200,000 $M^{-1}$ $cm^{-1}$ | between 504 nm and 512nm | 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 † | 578 nm | 1,960,000 $M^{-1}$ $cm^{-1}$ | 578 nm | S10467 |

* Qdot® nanocrystals are excitable (Ex, in nm) at any wavelength below their emission maxima (Em, in nm). For most practical applications, they should be exited at wavelengths below 405 nm.
† Multiple absorbance peaks.

## Before Starting

**Equipment Required**

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

**Materials Required but Not Provided**

- 100 to 125 µg of whole IgG antibody, preferably at a concentration of 2 to 4 mg/mL in a Tris-based buffer, free of carrier proteins and/or azide.
- Centrifuge tubes: 1.5-mL and 15-mL
- Distilled water ($dH_2O$)

**Caution**

- 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 www.lifetechnologies.com, before handling this reagent.
- The Qdot® conjugate contains cadmium and selenium in an inorganic crystalline form.
- 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. Antibody Concentration and/or 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 Components A, C, D, E, F, G, H, and J to ensure all material is at the bottom of the tubes.

### Wash Antibody Concentrator

**1.1** Add 450 µL of $dH_2O$ to the small antibody concentrator (Component B) and cap the device as shown in Figure 3, below.

**1.2** Centrifuge for 6 minutes at 5000 × *g*, 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 Exchange Buffer

**1.4** 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.

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

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

**1.7** Discard the flow through.

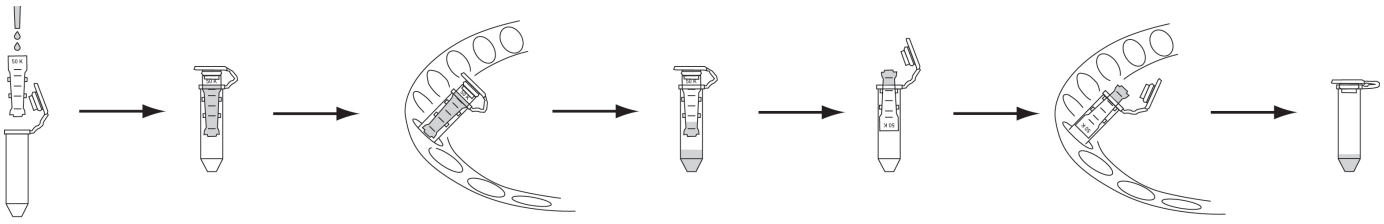
**1.8** Add 450 µL of antibody preparation buffer (Component A) to the small antibody concentrator (Component B) and centrifuge for 6 minutes at 5000 × *g*, ensuring that the cap strap and one membrane panel of the concentrator faces the center of the rotor.

**Note:** If antibody volume in concentrator is greater than 50 µL following Step 1.8, centrifuge for an additional 3 minutes at 5000 × *g* 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 3, below.

**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 3** Antibody concentration and/or buffer exchange



Fill (Step 1.4) | Spin (Step 1.6) | Recover (Step 1.9) | Collect (Step 1.10)

## Step 2. Modification of Antibody Carbohydrate Domain

**Time Required:** 4 hours, hands-off

### 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 4, below.

**2.2** Wrap the tube cap with Parafilm® laboratory film or similar and incubate for 4 hours at 37°C.

## Step 3. Azide Attachment

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

### Add GalT enzyme

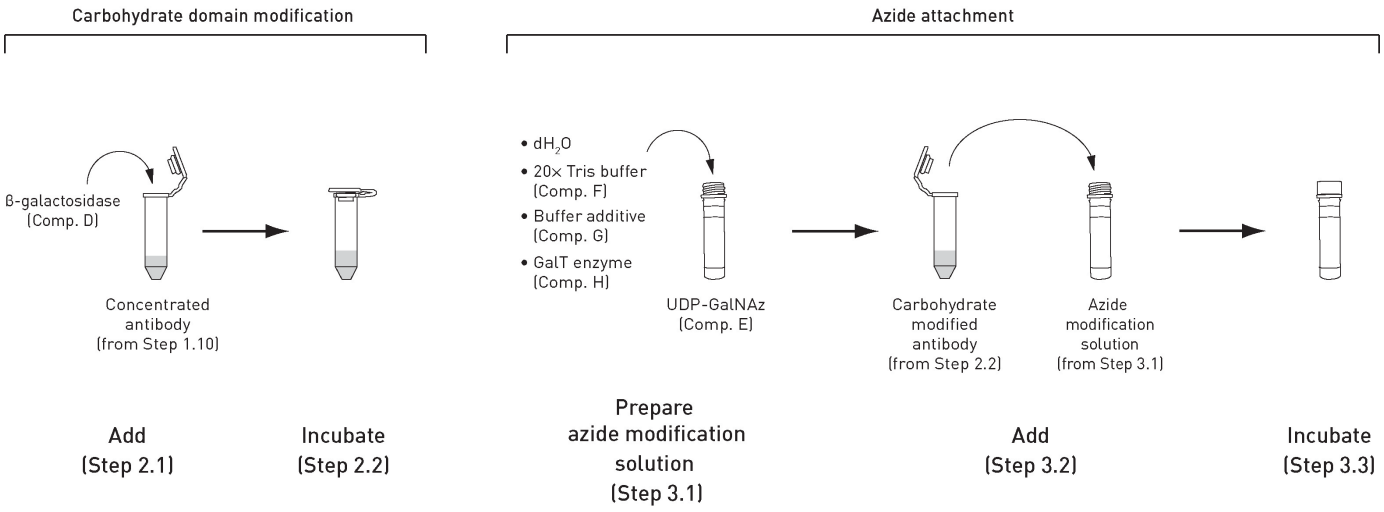
**3.1** Prepare the azide modification solution by adding the following components to the tube containing UDP-GalNAz (Component E), as shown in Figure 4, below:

- 75 µL of $dH_2O$
- 10 µL of 20× 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, and incubate overnight at 30°C.

**Figure 4** Modification of antibody carbohydrate domain and azide attachment



## Step 4. Purification and Concentration of Azide-Modified Antibody

**Time Required:** 1 hour

### Wash Antibody Concentrator

**4.1.** Prepare 10 mL of 1× Tris, pH 7.0 by adding 500 µL of 20× Tris, pH 7.0 (Component F) to 9.5 mL of $dH_2O$ in a 15-mL conical tube. Vortex briefly to mix.

**Note:** TBS may also be used for the purification and collection of the modified antibody (Steps 4.2 – 4.12). 20× Tris, pH 7.0 is provided for convenience.

**4.2** Remove the conical collection tube from the large antibody concentrator (Component I) as shown in Figure 5, below.

**4.3** Add 1 mL of 1× Tris, pH 7.0 to the large antibody concentrator (Component I) and centrifuge for 10 minutes at 1200 × *g*, ensuring that one membrane panel of the concentrator faces the center of the rotor.

**4.4** Discard flow through.

### Purify the Antibody

**4.5** Add 1.75 mL of 1× Tris, pH 7.0 and 250 µL of the azide modified antibody from Step 3.3 to the large antibody concentrator (Component I) as shown in Figure 5, below.

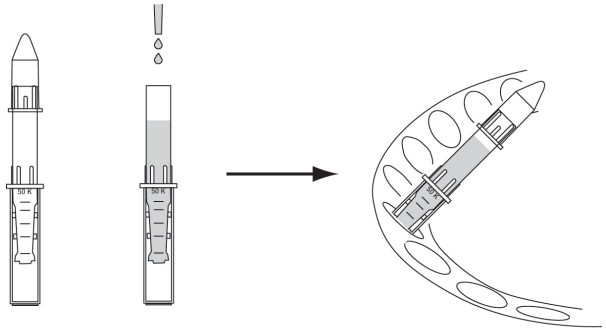
**4.6.** Centrifuge for 6 minutes at 1200 × *g*, ensuring one membrane panel of the concentrator faces the center of the rotor.

**4.7** Discard flow through.

**4.8** Add 1.8 mL of 1× Tris, pH 7.0 to the large antibody concentrator (Component I) and centrifuge for 10 minutes at 1200 × *g*, ensuring that one membrane panel of the concentrator faces the center of the rotor.

**4.9** Discard flow through and repeat Step 4.8 once more.

**Figure 5** Purification and concentration of azide-modifed antibody



Fill (Step 4.5) Spin (Step 4.6)

### Concentrate and Collect Antibody

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

**Note:** If antibody volume in concentrator is greater than 100 µL, centrifuge for an additional 5 minutes at 1400 × *g* or until the appropriate volume is achieved.

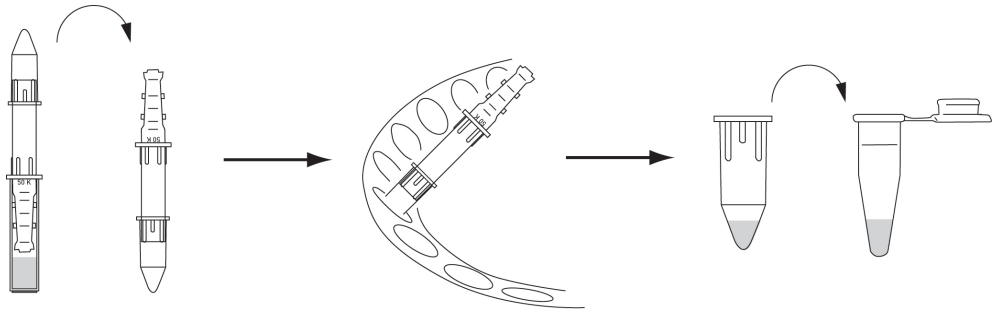
**4.11** Invert the antibody concentrator into the conical collection tube as shown in Figure 6, below.

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

**4.13** 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 antibody to 100 µL with 20× Tris, pH 7.0.

**Note:** At this stage, the antibody can be stored at 2–8°C for attachment of the DIBO-modified label at a later time.

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




## Step 5. Conjugation with DIBO-modified Label

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

### Add DIBO-modified Label

**5.1** 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).

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

**5.3** The antibody conjugate can now be stored at 2–8°C, protected from light (see **Antibody Conjugate Storage**, page 10) or optionally purified of excess antibody (Step 6, page 9).

## Step 6. Purification and Concentration of 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 may be colored, and this color may vary. This is normal and will not impact 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 Antibody

**6.1** Add 500 µL of $dH_2O$ to the purification concentrator (Component K) and centrifuge for 5 minutes at 1500 × *g*. Discard flow through.

**6.2** Add 150 µL of the labeled antibody conjugate from Step 5.2 to the purification concentrator (Component K) as shown in Figure 7, below.

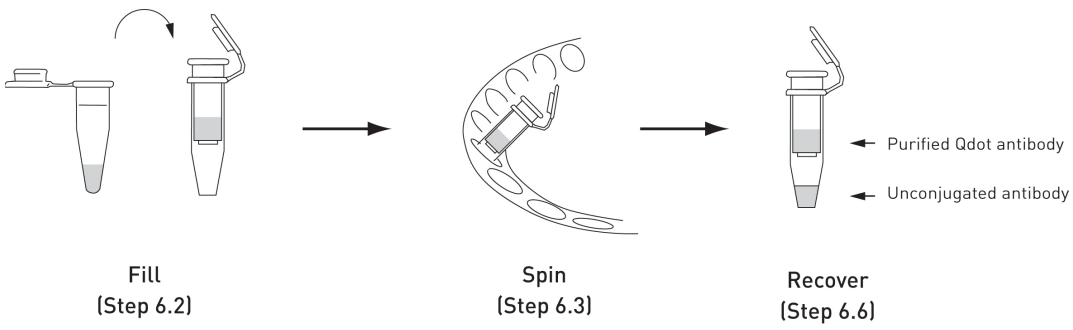
**6.3** Add buffer (TBS or PBS) to the purification concentrator to bring the volume to 500 µL and centrifuge for 10 minutes at 1500 × *g*. Discard flow through.

**6.4** Add 500 µL of buffer (TBS or PBS) to the purification concentrator and centrifuge for 10 minutes at 1500 × *g*.

**6.5** Discard flow through and repeat Step 6.4 once more.

**6.6** Collect the purified labeled antibody conjugate from the top of the concentrator and transfer to a new 1.5 mL centrifuge tube. The antibody conjugate can now be stored at 2–8°C, protected from light (see **Antibody Conjugate Storage**, page 10).

**Figure 7** Optional purification of conjugated antibody



## Storing and Using 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. Sodium azide or thimerosal can be added at this stage to a final concentration of 0.02% (w/v) for long term storage, if preferred.

**Antibody Conjugate Use**

The concentration of the label in the final preparation can be determined by measuring the optical density of the label at the specified wavelength and then using the formula $A = \varepsilon cL$, where A is the absorbance, ε is the molar extinction coefficient (Table 2, page 4), 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
$c = A/\varepsilon = 0.80/400{,}000 = 2\ \mu M$ of label, based on nanocrystal absorbance.

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

## Product List Current prices may be obtained from our website or from our Customer Service Department.

| Cat. no. | Product Name | Unit Size |
|---|---|---|
| S10449 | SiteClick™ Qdot® 525 Antibody Labeling Kit | 1 kit |
| S10450 | SiteClick™ Qdot® 565 Antibody Labeling Kit | 1 kit |
| S10451 | SiteClick™ Qdot® 585 Antibody Labeling Kit | 1 kit |
| S10469 | SiteClick™ Qdot® 605 Antibody Labeling Kit | 1 kit |
| S10452 | SiteClick™ Qdot® 625 Antibody Labeling Kit | 1 kit |
| S10453 | SiteClick™ Qdot® 655 Antibody Labeling Kit | 1 kit |
| S10454 | SiteClick™ Qdot® 705 Antibody Labeling Kit | 1 kit |
| S10455 | SiteClick™ Qdot® 800 Antibody Labeling Kit | 1 kit |
| S10467 | SiteClick™ R-PE Antibody Labeling Kit | 1 kit |

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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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