Qdot® Antibody Conjugation Kits

Catalog nos. A10197, Q22001MP, Q22021MP, Q22031MP, Q22041MP, Q22071MP, Q22011MP, Q22061MP

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage*	Stability
Reagent Components				
Qdot® nanocrystals (Component A)	1 vial containing 250 µL	4 µM	• 2–6˚C • DO NOT FREEZE	When stored as directed the kit is stable for at least 6 months
SMCC solution (Component B)	2 vials each containing 50 µL	10 mM solution in DMSO		
DTT (Component C)	50 µL	1 M		
2-mercaptoethanol (Component D)	50 µL	14.3 M		
Exchange buffer (Component E)	75 mL	50 mM MES, 2 mM EDTA, pH 6.0		
Separation media (Component F)	8 mL	suspension containing 20% ethanol		
Dye-labeled marker (Component G, lyophilized)	1 vial	NA		
Consumable Components (in a poly bag)				
Syringe (Component H)	1	NA	Room temperature	When stored as directed the kit is stable for at least 6 months
Column (Component I, for separation media),	2	NA		
Tubing (Component J)	1	NA		
Ultrafiltration device (Component K)	4	NA		
Centrifugation tube (Component L)	8	NA		
Desalting column (NAP™-5 column) (Component M)	4	NA		

*The entire kit can be stored under the conditions listed. For optimal storage conditions of individual kit components, refer to the labels on the vials. NA = Not applicable.

Number of Assays: Each kit provides sufficient reagents for two labeling reactions using ~300 µg of IgG antibody (or equivalent) in each reaction.

Introduction

The Qdot® Antibody Conjugation Kits, which contain amine-derivatized, PEG-coated nanocrystals and the amine–thiol crosslinker SMCC, allow you to conjugate your own antibodies to Qdot® nanocrystals (525, 565, 585, 605, 625, 655, 705, or 800 nm emission). The conjugation reaction can be completed in a few hours and is based on the fast and efficient coupling of thiols that are present in reduced antibodies to reactive maleimide groups present on the nanocrystals after SMCC activation. In addition to antibodies, other thiol-containing molecules can be coupled to Qdot® nanocrystals using these kits.

Each Qdot® Antibody Conjugation Kit contains sufficient reagents to perform two separate conjugation reactions of Qdot® nanocrystals to an antibody sample. The protocol in this manual describes a conjugation reaction starting with 300 µg of whole IgG, but the protocol works well with polyclonal Fab fragments. If conjugation of Qdot® nanocrystals to monoclonal antibodies is desired, optimization of the protocol (for example optimization of the antibody reduction) may be required. Refer to the Qdot® Antibody Kit Supplemental Information Guide at www. invitrogen.com/qdots for more information about nanocrystal conjugates and the use of Qdot® Antibody Conjugation Kits.

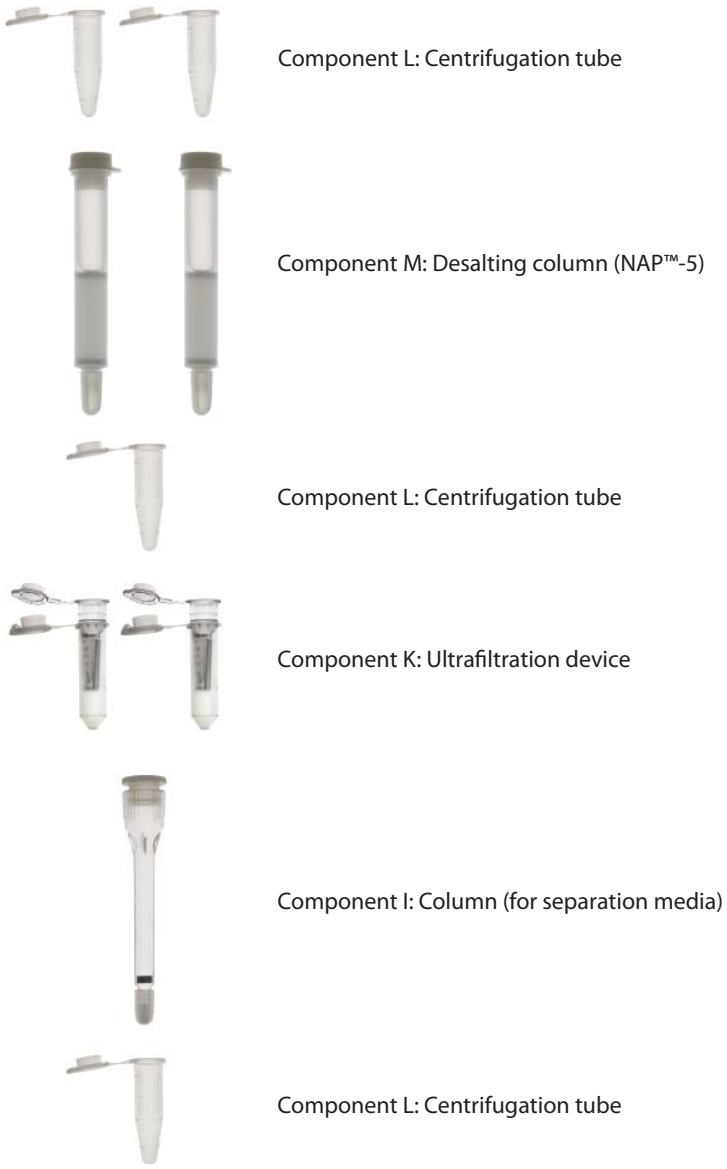

Figure 1. Order and number of consumable components used during a single Qdot® antibody conjugation procedure.

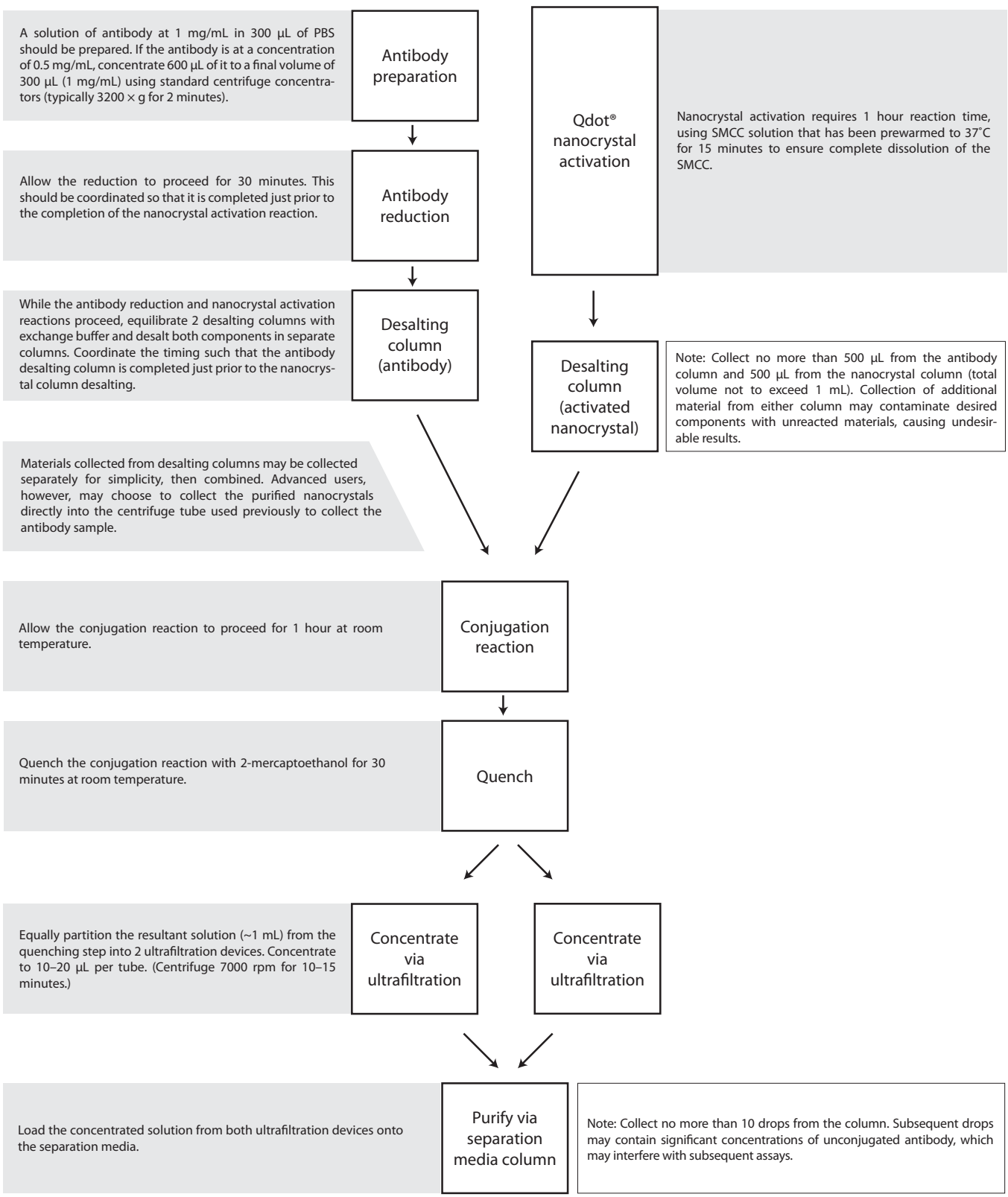

Figure 2. Workflow diagram of the Qdot® antibody conjugation procedure.

Figure 1 shows the consumable components of the kit that are used during the conjugation and the quantity of each required. Familiarize yourself with these components before you begin so that the correct one is used at the correct time. Figure 2 is a workflow diagram of the critical steps of the conjugation procedure for experienced users. In the interest of creating an abbreviated protocol diagram, some of the protocol details were omitted in Figure 2. Therefore, it is important to read the entire protocol supplied in this manual before beginning your conjugation reaction. Several steps require advance preparation, and successful conjugation requires execution of all steps in the right order.

Before You Begin

Please read the entire protocol before starting

Materials Required but Not Provided

- Phosphate buffered saline buffer (PBS; composition: 10 mM phosphate, 138 mM NaCl, 2.7 mM KCl, pH 7.2, Invitrogen Cat. no. 700 13-032)
- Benchtop microcentrifuge

Experimental Protocol

Preparing for the Conjugation Reaction

1.1 Thaw 1 **new** vial of SMCC solution (Component B) at 37°C for at least 15 minutes before use (see step 2.1 below).

1.2 Prepare 300 µL of a 1 mg/mL antibody solution in PBS by dilution or concentration. For example, if the antibody is at a concentration of 0.5 mg/mL, concentrate 600 µL to a final volume of 300 µL at 1 mg/mL using 50 KDa molecular weight cutoff centrifuge concentrators (not included in kit).

1.3 The first time you use a new Qdot® Antibody Conjugation Kit, add 40 µL of distilled water to supplied dye labeled marker (Component G) and mix. This makes enough dye labeled marker for two conjugation reactions. Store at 2–6˚C when not in use.

Activating Qdot® Nanocrystals

2.1 Pipette 14 µL of thawed SMCC solution into a centrifugation tube (Component L).

2.2 To the tube containing the SMCC, add 125 µL of Qdot® nanocrystals (Component A). Vortex briefly to mix.

Note: When activating Qdot® nanocrystals, always use SMCC from a new vial. After the aliquot of Qdot® nanocrystals is added to the aliquot of thawed SMCC, throw the rest of the vial of SMCC away. **Do not reuse SMCC**.

2.3 Incubate for 1 hour at room temperature to activate the nanocrystals.

2.4 Start the protocol **Reducing the Antibody Sample** (below) when there are 30 minutes remaining for the Qdot® activation reaction.

Note: Do not store activated Qdot® nanocrystals and reduced antibody. It is important to proceed with the desalting and conjugation reactions as soon as the Qdot® nanocrystals and antibody sample are ready.

Reducing the Antibody Sample

3.1 Pipette 300 µL of antibody at 1 mg/mL (see step 1.2) into a centrifugation tube (Component L).

3.2 Add 6.1 µL of DTT solution (Component C) to antibody and mix briefly.

3.3 Incubate for 30 minutes at room temperature.

3.4 Prepare the desalting columns while the antibody reduction step is proceeding.

Note: If desired, the desalting columns can be equilibrated with exchange buffer and capped before performing the **Activating the Qdot® Nanocrystals** step and **Reducing the Antibody Sample** step.

Equilibrating the Desalting Column

Prepare two desalting columns with exchange buffer prior to the end of the antibody reduction.

4.1 Label two desalting columns (Component M). Mark one "reduced antibody" and the other "activated Qdot® nanocrystals".

4.2 Remove top and bottom caps from both columns and just as the liquid in each column is approaching the top of the column gel bed, begin adding exchange buffer (see step 4.3).

4.3 Equilibrate each column gel bed with 10 mL (3 column volumes) of exchange buffer (Component E).

4.4 While there is still exchange buffer visible above the gel bed on each column, cap the bottom of each column and set aside until the antibody reduction is completed.

Desalting and Collecting the Reduced Antibody

5.1 Add 500 µL of water to a centrifugation tube (Component L) and mark the outside of the tube at the meniscus. Add another 500 µL of water and make a second mark on the outside of the tube corresponding to the new volume. Discard the water.

Note: This tube is used to collect the reduced antibody in step 5.6 and the activated Qdot® nanocrystals in step 6.4.

5.2 When the antibody reduction is completed, add 20 µL of dye labeled marker (prepared in step 1.3) to the reduced antibody.

5.3 Uncap the desalting column labeled "reduced antibody" and allow remaining exchange buffer to enter gel bed and as soon as it has done so, immediately add reduced antibody mixture (prepared in step 5.1) to the top of the gel bed.

5.4 Allow the reduced antibody mixture to completely enter the gel.

5.5 Add 1 mL of exchange buffer to the top of the gel bed to elute the antibody.

5.6 Begin collecting reduced antibody into a centrifugation tube (marked in step 5.1) when the first colored drop elutes; collect no more than 500 µL (to the lower marked line from step 5.1). Do not attempt to collect more than 500 µL as it may contain residual DTT that will interfere with the conjugation.

Desalting and Collecting the Activated Qdot® Nanocrystals

6.1 Uncap the desalting column labeled "activated Qdot® nanocrystals" allow remaining exchange buffer to enter gel bed and as soon as it has done so, immediately add the activated Qdot® nanocrystals (from step 2.3) to the top of the gel bed.

6.2 Allow the activated Qdot® nanocrystals mixture to completely enter the gel.

6.3 Add 1 mL of exchange buffer to top of gel bed to elute the Qdot® nanocrystals

6.4 When the first drop of colored material elutes from the column, begin collecting directly into the centrifugation tube containing the reduced and desalted antibody.

6.5 Stop collecting when the final volume reaches 1 mL (up to the top line marked in step 5.1; 500 µL of activated Qdot® nanocrystals).

6.6 Mix briefly.

Conjugation Reaction

7.1 Allow the reduced antibody and activated Qdot® nanocrystals to react for 1 hour at room temperature.

7.2 During the conjugation reaction or quenching step, prepare the separation column (see **Preparing the Separation Column**).

Quenching the Conjugation Reaction

8.1 Prepare a 10 mM working solution of 2-mercaptoethanol just before using. To do this, add 3 µL of 2-mercaptoethanol (Component D) to 4 mL distilled water.

8.2 Add 10 µL of diluted 2-mercaptoethanol to the conjugation reaction from step 7.1.

8.3 Incubate for 30 minutes at room temperature.

Preparing the Separation Column

During the conjugation or quenching step, prepare the separation column. The separation media (Component F) is supplied as a suspension containing 20% ethanol as a preservative.

9.1 Remove and save the top and bottom column caps from a new separation column (Component I).

9.2 Suspend the separation media (Component F) in the bottle with gentle shaking or vortexing. Ensure the media is fully suspended (some may be stuck to the underside of the cap) before starting column preparation.

9.3 Mark the column with two lines, one at 45 mm above the top of the frit, and a second at

55 mm above the frit.

Note: These two marks serve to indicate how much suspended separation media to add and, consequently, the height of the packed gel bed. A uniform suspension of separation media added to the 55 mm mark should settle into a packed gel bed about 45 mm high.

9.4 After ensuring that the separation media is a uniform suspension, load media into the column with a 1 mL pipette to the second line at 55 mm mark. The column begins to drip at the bottom.

9.5 Gently add 0.5 mL distilled water to top off the gel while maintaining a level bed surface.

9.6 Attach one end of the tubing (Component J) to the tip of the column, and attach the other end to the syringe (Component H) that has the plunger completely depressed.

9.7 By **slowly** drawing the syringe plunger out, withdraw the solvent from the column. Do not allow the solvent to drain below the top of the gel bed.

9.8 As the solvent level drops to near the top of the settled gel bed, fill the column with PBS pH 7.2 and, using the syringe, draw the PBS level down to just above the top of the gel bed. Repeat this PBS fill and drain two more times, using the syringe to draw the PBS through.

9.9 When you have drawn the PBS from the last fill down to a level 2 to 3 mm above the top of the settled gel bed line, remove the syringe and replace the bottom and top caps.

Concentrating the Sample

10.1 Split the volume of the quenched conjugation reaction (from step 8.3) into two ultrafiltration devices (Component K).

10.2 Concentrate each half reaction to ~20 µL by centrifuging at 4000 × g for approximately 10 to 15 minutes. This corresponds to ~7,000 rpm in most benchtop microcentrifuges.

10.3 If the volume is larger than 20 µL after this initial centrifugation, continue centrifuging for another 5 minutes.

Separating the Conjugated Antibody from Unconjugated Antibody

11.1 Uncap the separation column (from step 9.9) and allow the PBS to elute by gravity so that it is just at the top of the column bed.

11.2 Immediately add to the gel bed the concentrated conjugate reaction from the two ultrafiltration devices (~40 µL total volume).

11.3 Allow the conjugate to enter gel and then gently add 50 µL of PBS pH 7.2 and allow that to run into the gel bed.

11.4 Gently fill the reservoir above the column with PBS and allow the sample to elute by gravity. Visually monitor the "dead space" between the frit and the column tip.

11.5 **When color appears in the "dead space," collect the first ten drops only** of colored conjugate in a centrifugation tube (Component L). **Do not collect more than the first ten drops from the column**. Subsequent drops contain unconjugated antibody that will interfere with the application the conjugate is used for.

Note: The second colored band above the Qdot® conjugate comes from the dye marker added

to the antibody reduction reaction. This is **NOT** an indication of where the free antibody runs as free antibody will elute much closer to the actual conjugate.

11.6 Add sodium azide to the collected conjugate at a final concentration of 0.01% (w/v) to serve as a preservative, if desired.

11.7 Store the conjugate at 4°C. **Do not freeze** the conjugate.

Conjugate collected from the separation column is typically in the 1 to 2 micromolar range. If desired, the conjugate concentration can be determined by measuring the optical density of the conjugate 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), c is the molar concentration, and L is the pathlength.

For example, for a Qdot® 655 antibody conjugate, if material eluting from the final column has A = 0.65 measured in a cuvette with 1 cm pathlength, then $c = A/\varepsilon = 0.65/800{,}000 = 0.812$ μM conjugate, based on nanocrystal absorbance.

Determine optimal working concentrations by performing a titration series for the application of interest. We typically use a conjugate concentration of 10 nM for immunocytochemistry applications. Recommended protocols on use of Qdot®antibody conjugates are available from probes.invitrogen.com.

Table 2. Extinction coefficients and measurement wavelengths for Qdot® nanocrystals..

Product	Extinction Coefficient (ε)	Measurement Wavelength
Qdot® 525 nanocrystals	200,000 M^{-1} cm^{-1}	At the highest absorbance value between 504 and 512 nm
Qdot® 565 nanocrystals	300,000 M^{-1} cm^{-1}	At the highest absorbance value between 540 and 556 nm
Qdot® 585 nanocrystals	400,000 M^{-1} cm^{-1}	At the highest absorbance value between 567 & 575 nm
Qdot® 605 nanocrystals	650,000 M^{-1} cm^{-1}	At the highest absorbance value between 596 & 604 nm
Qdot® 625 nanocrystals	500,000 M^{-1} cm^{-1}	At the highest absorbance value between 605 & 612 nm
Qdot® 655 nanocrystals	800,000 M^{-1} cm^{-1}	638 nm exactly
Qdot® 705 nanocrystals	1,700,000 M^{-1} cm^{-1}	550 nm exactly
Qdot® 800 nanocrystals	1,700,000 M^{-1} cm^{-1}	550 nm exactly

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

Cat. no.	Product Name	Unit Size
Q22041MP	Qdot® 525 Antibody Conjugation Kit	1 kit
Q22031MP	Qdot® 565 Antibody Conjugation Kit	1 kit
Q22011MP	Qdot® 585 Antibody Conjugation Kit	1 kit
Q22001MP	Qdot® 605 Antibody Conjugation Kit	1 kit
A10197	Qdot® 625 Antibody Conjugation Kit.	1 kit
Q22021MP	Qdot® 655 Antibody Conjugation Kit	1 kit
Q22061MP	Qdot® 705 Antibody Conjugation Kit	1 kit
Q22071MP	Qdot® 800 Antibody Conjugation Kit	1 kit

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