Qdot® Streptavidin Conjugates

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage	Stability
Qdot® streptavidin conjugate	200 µL or 50 µL	1 µM solution in 1 M betaine, 50 mM borate, pH 8.3 with 0.05% sodium azide*	• 2–6°C • Do not freeze	When stored as directed, product is stable for at least 6 months.

*Betaine acts as a cryoprotectant during shipping and does not affect the fluorescence of Qdot® conjugates.

Approximate fluorescence excitation/emission maxima: See Figure 3.

Introduction

Structure of Qdot® Nanocrystals

The Qdot® streptavidin conjugate is made from a nanometer-scale crystal of a semiconductor material (CdSe), which is coated with an additional semiconductor shell (ZnS) to improve the optical properties of the material. These materials have a narrow, symmetric emission spectrum with the emission maximum near 525 nm (Q10141MP), 565 nm (Q10131MP), 585 nm (Q10111MP), 605 nm (Q10101MP), 625 nm (A10196), 655 nm (Q10121MP), 705 nm (Q10161MP), or 800 nm (Q10171MP). The Qdot® 705 and Qdot® 800 streptavidin conjugates, which include CdSeTe, are made in a similar fashion. This core-shell material (Figure 1A) is further coated with a polymer shell that allows the materials to be conjugated to biological molecules and to retain their optical properties. This polymer shell is directly coupled to streptavidin (Figure 1B). The Qdot® streptavidin conjugate is the size of a large macromolecule or protein (~15–20 nm).

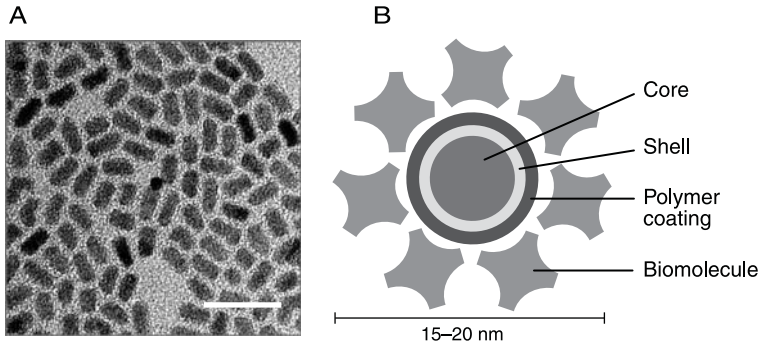

Figure 1. A. Transmission electron microscope image of core-shell Qdot® nanoparticles at 200,000x magnification. Scale bar = 20 nm. **B.** Schematic of the overall structure of a Qdot® streptavidin conjugate. The layers represent the distinct structural elements of the Qdot® nanocrystal conjugates, and are roughly to scale.

Optical Properties

The optical properties of Qdot® conjugates are different than those of typical dye molecules. The color of light that the Qdot® nanocrystal emits is strongly dependent on the particle size, creating a common platform of labels from the green to the red, all manufactured from the same underlying semiconductor material (see *Bibliography*, references 1–11 in the *Appendix*). The size of Qdot® nanocrystals are tightly controlled in the production process, resulting in materials with narrow and symmetric emission spectra, that are extremely bright and photostable. While the fluorescence emission from the Qdot® 705 and Qdot® 800 streptavidin conjugates are broader than the other Qdot® conjugates, the fluorophores have similar intensities and photostabilities. Note that the 705 and 800 nm quantum dot emission cannot be seen by eye, but is easily detected by many cameras and detectors. These properties are exploited in a variety of immunofluorescence techniques, and can result in substantially better results than are attainable with conventional immunofluorescent labels (see *Bibliography*, references 12–18). Though these materials are compatible with a number of standard immunofluorescent techniques, there are some novel aspects of their chemistry and detection that require careful consideration to attain optimal assay results.

Biological Activity

The surface chemistry dictates many of the important properties of the Qdot® nanocrystal in a biological experiment. The surface has been prepared to have a low nonspecific signal when incubated with samples in a variety of aqueous buffers. Qdot® nanocrystals have been coupled to streptavidin directly through an active ester coupling reaction.[19] This yields a material with streptavidin covalently attached on the surface (typically 5–10 streptavidins/Qdot® conjugate), which results in Qdot® streptavidin conjugates with high specific biological activity. The probes should generally be used as if there were one streptavidin per Qdot® nanocrystal. Though one quantum dot is capable of bridging multiple antigens through a biotinylated IgG, the dominant binding mode is one Qdot® conjugate per analyte if the assay is carried out at a saturating concentration. (Figure 2.)

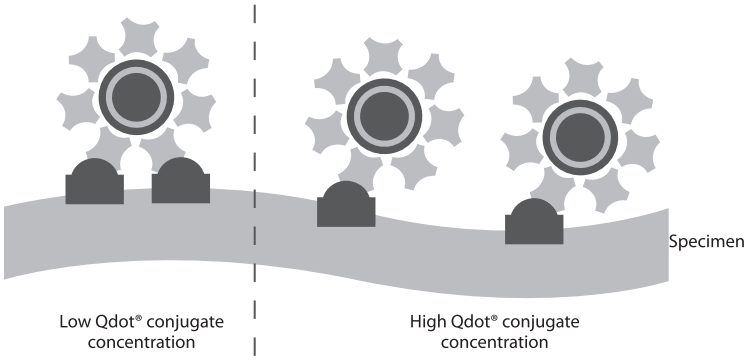

Figure 2. Impact of working at the appropriate concentration range for Qdot® streptavidin conjugates. Due to the multivalency of the conjugates, use of conditions below the appropriate saturation concentration may result in artificially reduced signals due to antigen bridging with a single quantum dot conjugate.

Spectral Characteristics

Typical fluorescent dyes have excitation and emission spectra with a relatively small Stokes shift, which means that the optimal excitation wavelength is close to the emission peak. Filter sets used with fluorescent dyes reflect this characteristic.[20] Qdot® nanocrystals have absorbance spectra that increase dramatically to the blue of the emission (Figure 3). These unique spectral properties are due to the semiconductor that makes up the core of the Qdot® conjugates, which gives rise to both the absorbance and emission properties of the materials (see *Bibliography*, references 1–11). Despite the broad absorbance, the emission wavelength is independent of the excitation wavelength; so whether exciting at 633 nm or at 400 nm, the shape of the emission remains the same, while the intensity is approximately 11-fold

higher with 400 nm excitation. The absorbance and excitation at shorter wavelength, with fixed emission for the material results in a large "apparent Stokes shift" which improves sensitivity by reducing auto-fluorescence, and greatly simplifies the multiplexed detection of several Qdot® conjugates. See *Appendix 3* for extinction coefficients at common excitation wavelengths of the different materials.

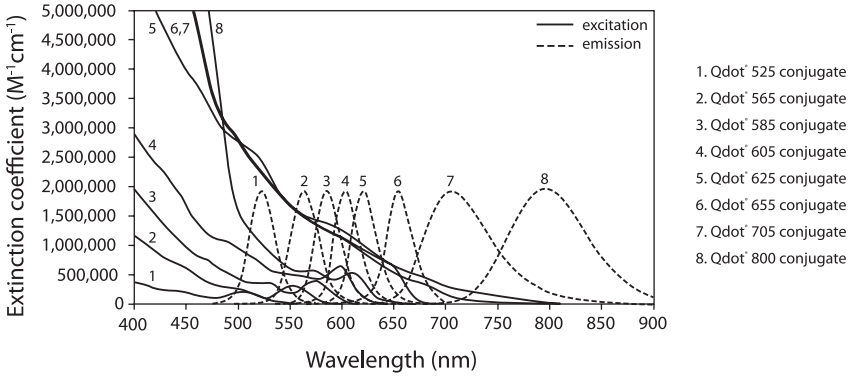

Figure 3. Typical absorption and emission spectra of Qdot® 525 streptavidin conjugate (1), Qdot® 565 streptavidin conjugate (2), Qdot® 585 streptavidin conjugate (3), Qdot® 605 streptavidin conjugate (4), Qdot® 625 streptavidin conjugate (5), Qdot® 655 streptavidin conjugate (6), Qdot® 705 streptavidin conjugate (7), Qdot® 800 streptavidin conjugate (8).

Optical Filter Selection

To achieve the optimal signal from Qdot® streptavidin conjugates, we recommend using Qdot® optimized filter sets that are available from Omega Optical, Semrock, or Chroma Technology Corporation (see *Appendix 2* for details).

The Qdot® streptavidin conjugate can also be viewed through some standard filter sets,

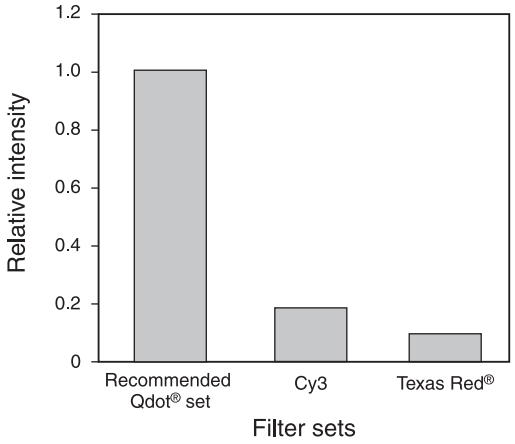

Figure 4. Detection of Qdot® conjugates on tissue sections with recommended and standard filter sets. Mouse kidney sections were stained with Qdot® 605 streptavidin conjugate, and then images were collected on a Nikon epi-fluorescence microscope in 16 bit capture mode. The mean fluorescence of positively stained samples was extracted using Scion Image software. The recommended Qdot® filter set included a 460 nm short pass exciter, a 475 nm dichroic, and a 605/20 nm band pass emitter. The Cy3 filter set included a 545/30 nm exciter, a 570 nm dichroic, and a 610/75 nm emitter. The Texas Red® filter set included a 560/40 nm exciter, a 595 nm dichroic, and a 630/60 nm emitter.

albeit with lower detection efficiency and reduced brightness. For example, three Omega Optical standard filter sets capable of detecting Qdot® 705 conjugates are XF140-2 (Alexa Fluor® 633 & Alexa Fluor® 647), XF70 (Alexa Fluor® 660 & Cy5), and XF141-2 (Cy5.5). Visualization of Qdot® conjugates using a custom filter set is preferred because excitation and detection is less efficient using filters that have not been selected specifically for use with Qdot® conjugates. Using a custom filter set, Qdot® 605 streptavidin conjugate signal is approximately five times as bright as it is using the TRITC filter set, and approximately ten times brighter than it is using the Texas Red®/Cy3.5 filter set (Figure 4). Qdot® optimal filters and standard filter sets are available from many different filter manufacturers. *Appendix 2* illustrates some common filter sets and the optimal filter set recommendations for the available Qdot® streptavidin conjugates. Use of the optimal filter set is critical for attaining optimal signal and sensitivity in your experiments. For detailed technical notes and examples of how to set up specific instruments to detect Qdot® conjugates optimally, visit www.invitrogen.com.

Before You Begin

Qdot® nanocrystals have chemical and optical properties that provide significant advantages over conventional fluorophores in both sensitivity and stability in immunofluorescent labeling of cells and tissue sections. We have conjugated streptavidin to Qdot® nanocrystals to allow use of the materials in a wide variety of labeling applications. Biotinylated antibodies can be obtained or easily prepared using available reagents without substantial disruption of the specificity or affinity of the native antibody. These antibodies can be specifically bound to the biological specimen and then detected through a second round of staining with a Qdot® streptavidin conjugate.

Note: The 705 and 800 nm emission cannot be seen by eye, but is easily detected by many cameras and detectors.

General Considerations

Buffer compatibility

The Qdot® streptavidin conjugates have stable emission in a number of distinct buffers, across a range of pH conditions. At working concentrations, the quantum yield and colloidal dispersion of these materials has been found to be remarkably stable across pH 6–9 (not investigated outside this range) in Tris, HEPES, phosphate, and borate buffers. The Qdot® streptavidin conjugates are stable and non-aggregated in buffered NaCl up to 200 mM at working concentrations. Higher salt concentrations result in microscopic aggregates, but do not appear to cause bulk precipitation of the materials at working dilutions. In addition, a number of surfactants and additives such as Tween 20, Triton® X-100, Pluronic F68, NDSB 201, and EDTA, among others have been shown to maintain the fluorescence when used at 0.05% concentration. In contrast, gelatin and dextran sulfate were found to promote aggregation of the Qdot® 605 streptavidin conjugate at 0.05% concentration, and should be avoided in labeling applications. In general, we recommend storage of Qdot® conjugates at the concentration at which it is shipped, rather than at a high dilution. Storage of Qdot® conjugates at working dilution over longer periods of time may result in substantial performance degradation. While we have not characterized the stability of all Qdot® streptavidin conjugates in all of these conditions, we anticipate similar levels of stability across the range of product colors.

Controls

If you are using the Qdot® conjugates or the labeling protocol for the first time, we recommend including a positive labeling control. For example, an optional control for Qdot® goat-anti-mouse secondary antibodies is anti-OxPhos Complex V Inhibitor Protein (Invitrogen Cat. no. A21355) which targets mitochondria. Other antibodies used in the optimization of this protocol and suitable as positive controls include: rabbit-anti-giantin

(Covance, Cat. no. PRB-114C), rabbit anti-AIF (Cell Signaling, Cat. no. 4642), mouse anti-ki-67 (Ventana Medical Systems, Cat. no. 790-2910), mouse anti-alpha tubulin (Sigma, Cat. no. T6074), rabbit anti-alpha tubulin (Affinity Bioreagents, Cat. no. PA1-20988), mouse anti-nucleosome (BD Pharmingen, Cat. no. 51-80591N), and mouse anti-nucleolin (Invitrogen, Cat. no. 39-6400; Stressgen, Cat. no. KAM-CP100).

Antigen labeling with Qdot® conjugates

Detecting cellular targets with Qdot® streptavidin conjugates can be performed individually by using a single Qdot® streptavidin conjugate with one primary antibody, or multiplexed by using a combination of primary antibodies and various Qdot® nanocrystal colors. Golgi, tubulin, mitochondrial, peroxisome, nucleolin, nucleosome, and ki-67 targets have been validated with this labeling protocol (for a complete list of primary antibodies validated, see above). Other targets and cell lines, however, may require further optimization of this protocol. For example, reducing the fixation time may improve cell penetration and conjugate access for some targets as may increasing the concentration of permeabilization reagent or incubation time with the permeabilization buffer.

Qdot® nanocrystal toxicity

We have not investigated the toxicity of the Qdot® streptavidin conjugate. The materials are provided in a solution which is ~2 mM total Cd concentration; however, the CdSe core is encapsulated in a shell of ZnS and the polymer shell, which may help prevent formation of free Cd. We have demonstrated the utility of these materials in a variety of live-cell *in vitro* labeling experiments, but do not have systematic data on the toxicity of the materials to humans, to animals, or to cells in culture.

FRET or close-proximity quenching

We have not systematically investigated the energy transfer properties of the Qdot® nanocrystals, though they may have useful properties as energy transfer donors and acceptors. We have investigated the fluorescence of Qdot® 605 streptavidin conjugates which are coupled to each other through a bis-biotin linker, and found that the emission intensity of the materials was unperturbed at any concentration of biotin cross-linker. These results suggest that the interparticle quenching of these Qdot® conjugates is negligible. Recent published literature indicates that Qdot® nanocrystals can be used as energy acceptors in time-resolved FRET (TR-FRET) studies.[21]

Disposal of Qdot® Conjugate

The Qdot® conjugate contains cadmium and selenium in an inorganic crystalline form. Dispose of the material in compliance with all applicable local, state, and federal regulations for disposal of these classes of material. For more information on the composition of these materials, consult the Material Safety Data Sheet.

Materials Required but Not Provided

- 10X PBS, pH 7.4 (phosphate buffered saline, Invitrogen Cat. no. 70011-044)
- Fixative: 4% formaldehyde in PBS
- Permeabilization buffer: 0.25% Triton® X-100 in PBS
- Wash buffer: 1X PBS, pH 7.4
- Endogenous Biotin Blocking Kit (Invitrogen Cat. no. E21390)
- Blocking buffer: 6% BSA (bovine serum albumin) and 10% normal serum in PBS
- Primary antibody, biotinylated primary antibody, or other biotinylated protein
- Biotinylated secondary antibody if using non-biotinylated primary antibody
- Secondary incubation buffer: 6% BSA in PBS
- Dehydration solutions: Prepare in containers suitable for a dehydration series ethanol/water dilutions (v/v) of 30%, 50%, 70%, and 90%, as well as 100% ethanol and 100% toluene
- Mounting reagent: Cytoseal™ 60 (Richard-Allan Scientific, Cat. no. 8310-16) is recommended

Preparing Solutions

The amount of solutions prepared as described below is sufficient to process approximately 20 coverslips.

Fixative: 4% formaldehyde in PBS

Prepare 40 mL of fixative **fresh** by combining 10 mL of formaldehyde (ultrapure, methanol-free, 16% formaldehyde solution, Polysciences, Inc. Cat. no. 18814) and 4 mL of 10X PBS, pH 7.4 (Invitrogen Cat. no. 70011-044) with 26 mL of distilled water. Mix well.

Permeabilization buffer: 0.25% Triton® X-100 in PBS

Prepare 40 mL of Permeabilization buffer by adding 100 µL of Triton® X-100 (Sigma, Cat. no. T9284) to 40 mL of 1X PBS. Stir until the Triton® X-100 goes into solution. Store any remaining Permeabilization buffer at 4°C for up to a week.

Wash buffer: 1X PBS, pH 7.4

Prepare 2 L of 1X PBS by combining 200 mL of 10X PBS, pH 7.4 (Invitrogen Cat. no. 70011-044) and 1.8 L of distilled water. Mix well.

Blocking buffer: 6% BSA/10% normal serum in PBS

To prepare 50 mL Blocking buffer, add 3 g of BSA (RIA grade, Fraction V, minimum 96%, Sigma Cat. no. A-7888), 5 mL of normal serum from the host species of the secondary antibody (preferably heat inactivated at 56°C for 1 hour), and 5 mL of 10X PBS, pH 7.4 to 40 mL distilled water. Mix well until the BSA is completely dissolved and adjust the volume to 50 mL with distilled water. Mix well with gentle rocking or stirring. If storing the blocking buffer, add sodium azide to a final concentration of 0.02% and store at 4°C.

Note: Avoid using blocking buffers with casein as casein can cause quenching of Qdot® conjugates.

Primary antibody, biotinylated primary antibody, or other biotinylated protein

Dilute the primary antibody, biotinylated primary antibody, or other biotinylated protein in Blocking buffer at the concentration recommended by the manufacturer.

Note: Briefly centrifuge the primary antibody prior to use. You may need to titrate the primary antibody concentration in preliminary experiments to achieve optimal target labeling.

Biotinylated secondary antibody (required if using non-biotinylated primary antibody)

Dilute the biotinylated secondary antibody in Blocking buffer at the concentration recommended by the manufacturer.

Secondary incubation buffer: 6% BSA in PBS

To prepare 50 mL Secondary incubation buffer, add 3 g of BSA (RIA grade, Fraction V, minimum 96%, Sigma Cat. no. A-7888) and 5 mL of 10X PBS, pH 7.4 to 40 mL of distilled water. Mix well until BSA is completely dissolved and adjust the final volume to 50 mL with distilled water. Mix well with gentle rocking or stirring. If storing the Secondary incubation buffer, add sodium azide to a final concentration of 0.02% and store at 4°C.

Dehydration solutions

Prepare in containers suitable for a dehydration series with ethanol/water dilutions (v/v) of 30%, 50%, 70%, and 90%, as well as 100% ethanol and 100% toluene.

Preparing Qdot® Streptavidin Conjugate

Do not vortex the Qdot® streptavidin conjugate vial. Prepare the required amount of the diluted Qdot® streptavidin conjugate needed for the experiment on the day of use. You will need 40–200 µL Qdot® streptavidin conjugate per coverslip depending on your protocol and the type of humidity chamber you use.

1.1 Centrifuge the Qdot® streptavidin conjugate vial at 5000 × g for 3 minutes prior to use. Use only the supernatant and discard any pellet.

1.2 Dilute the conjugate by adding 2 µL of the stock (1 µM) conjugate to 100 µL Secondary incubation buffer immediately prior to use to obtain the Qdot® streptavidin conjugate concentration of 20 nM (you may use between 10 nM and 40 nM Qdot® streptavidin conjugate final concentration).

1.3 Use the diluted Qdot® streptavidin conjugate immediately for the current experiment. **Do not** store any diluted Qdot® streptavidin conjugate.

Preparing Cells

Culture cells in the appropriate medium to the desired confluency and physiological state (typically 1–2 days for HeLa cells). Make sure the cells are below 80% confluency at the time of fixation, depending on experimental requirements and the imaging method.

Experimental Protocols

Cell Labeling Protocol

Please read entire protocol before starting.

This protocol was validated with HeLa and NIH 3T3 cells, but can be adapted for any adherent mammalian cell types. For tissue labeling protocols, refer to the Qdot® Conjugates Protocol Handbook (mp19029) available for downloading from www.invitrogen.com. Perform all steps of the procedure at room temperature. For a flowchart of the labeling protocol, see Figure 5.

The optimal signal-to-background ratio is obtained from an assay that has been optimized with respect to all of the probe concentrations. Excess concentrations of the primary or secondary antibodies can cause increases in background staining. The optimal concentrations of these reagents can be determined by testing the signal-to-background ratio of control and positive samples that are treated with a dilution series of each antibody, and then stained under typical conditions.

Fixation and Permeabilization

2.1 Rinse cells briefly in 1X PBS prewarmed to 37°C.

2.2 Fix cells in Fixative for 15 minutes. Pour off or aspirate the solution.

2.3 Wash cells 3 times with 1X PBS. If cells are grown on coverslips in a large dish (i.e., 100 mm petri dish), add sufficient volume of 1X PBS to completely cover the specimen (~5 mL for a 100 mm petri dish), swirl gently, pour off or aspirate the solution and repeat. If cells are grown on coverslips in a smaller container (i.e., 6-well plate), wash cells 3 times for 5 minutes each by adding enough 1X PBS to completely cover the specimen. After 5 minutes, pour off or aspirate the solution.

2.4 Permeabilize the specimen with Permeabilization buffer for 15 minutes. Pour off or aspirate the solution.

2.5 Repeat step 2.3.

Note: Changing fixation or permeabilization times, or reagent concentrations may be needed for achieving labeling of certain targets.

Target Labeling and Detection

All incubations are performed in a humidity chamber at room temperature (see **Note**, below). Avoid specimen drying as this can cause high levels of non-specific background and autofluorescence. At the end of each step, carefully remove or blot excess solution from the sample before moving to the next step.

Note: A simple humidity chamber prevents labeling reagent concentration changes due to

evaporation during incubations. To make a simple humidity chamber, place a piece of filter paper in a Petri dish and saturate the filter paper with water. Place a piece of laboratory film on the filter paper and place the coverslip with the solution on the laboratory film. During incubations, place a lid on the Petri dish. Gentle agitation during incubation steps is optional.

2.6 Perform blocking of endogenous biotin with the Endogenous Biotin Blocking Kit (Invitrogen, Cat. no. E21390) as follows. The kit contains two reagents supplied in a ready-to-use format.

a) Incubate in Component A for 30 minutes.

b) Wash 3 times with 1X PBS for 5 minutes each.

c) Incubate in Component B for 30 minutes.

d) Wash 3 times with 1X PBS for 5 minutes each.

2.7 Add Blocking buffer and incubate for 1 hour. Pour off or aspirate the blocking buffer.

2.8 Incubate for 1 hour with primary antibody, biotinylated primary antibody, or other biotinylated protein, diluted in blocking buffer. Pour off or aspirate the solution.

2.9 Wash 3 times with 1X PBS for 5 minutes each.

Note: If using a biotinylated secondary antibody, repeat step 2.8 with the biotinylated antibody and perform wash step 2.9 before proceeding to the next step.

2.10 Incubate for 1 hour with Qdot® streptavidin conjugate, diluted to an optimal concentration (titrate between 10 nM and 40 nM for optimal results) in secondary incubation buffer. Pour off or aspirate the solution.

2.11 Wash 3 times with 1X PBS for 5 minutes each.

2.12 **Optional:** If counterstaining is necessary, most counterstain procedures may be performed at this point, followed by necessary wash steps.

Note: UV excitation of DAPI (Invitrogen, Cat. no.D1306) resulting in emission around 600 nm has been observed, and may not be appropriate for use with all Qdot® nanocrystals. Several other nuclear counterstain options are available in a kit form with optimized protocols for use with Qdot® conjugates such as SelectFX® Nuclear Labeling Kit (Invitrogen, Cat. no. S33025), SYTOX® Green nucleic acid stain (Invitrogen, Cat. no. S7020), 7-aminoactinomycin D (7-AAD) (Invitrogen, Cat. no. A1310), propidium iodide (Invitrogen, Cat. no. P3566), and TO-PRO®-3 iodide (Invitrogen, Cat. no. T3605).

2.13 Dehydrate the specimen by submerging the coverslip sequentially for 5 seconds in 30%, 50%, 70%, and 90% ethanol/water, twice in 100% ethanol, once in 100% toluene, and the final dip in 100% toluene for 10 seconds.

When transferring the coverslip from the last toluene wash to the mounting medium, blot any excess toluene with a laboratory wipe, but allow a sheen of toluene to remain on the coverslip surface. **Do not** allow the residual toluene on the coverslip to completely evaporate prior to mounting, as this can lead to cell morphology damage and/or high background.

2.14 Mount using one drop of Cytoseal™ 60 and allow the specimen to cure at least 4 hours before imaging. For optimal performance, image within 24 hours.

Imaging Guidelines

For optimal imaging of Qdot® streptavidin conjugates, including reduced spectral bleedthrough effects in multi-color applications, use filter sets optimized for the excitation and emission of the Qdot® conjugates in use. These filters are available from Omega Optical, Semrock, or Chroma Technology Corporation (see *Appendix 2* for details). For additional information, visit probes.invitrogen.com/products/qdot.

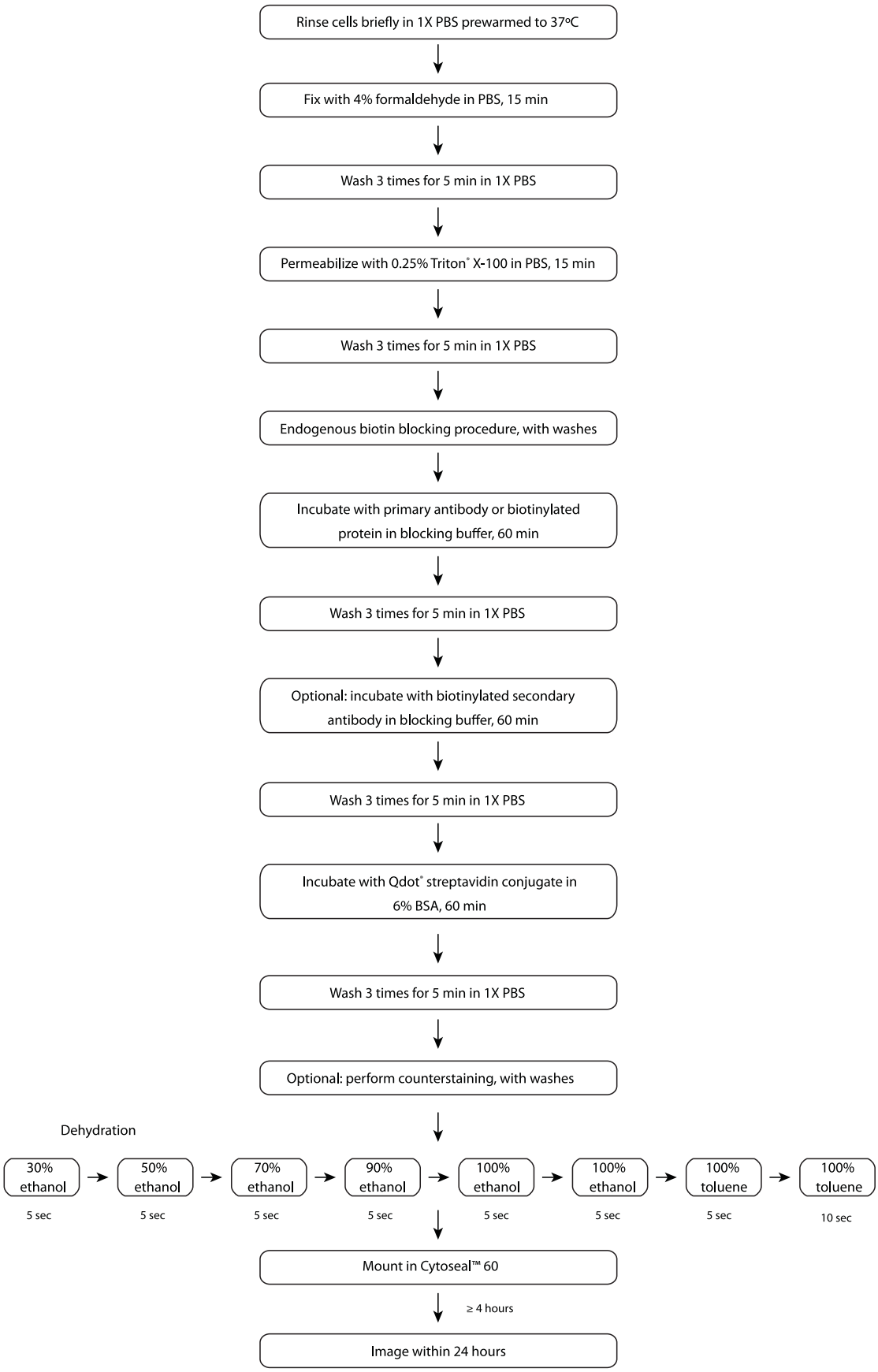

Figure 5. Cell labeling flowchart.

Appendix 1: Troubleshooting Guide

The properties of Qdot® conjugates are different from fluorescent dyes and may require slight modifications to current protocols. We've included this section to help with some specific issues that may arise while using these materials.

No Signal

Setup suitability

Make sure that you are using an appropriate filter set to detect the signals. See *Appendix 2* for a list of appropriate and optimal filters for the Qdot® conjugates. Contact Technical Support (probestech@invitrogen.com) for more details on particular filter set requirements.

Qdot® conjugate luminosity

Qdot® conjugates normally fluoresce brightly under a hand-held ultraviolet lamp (long wave, such as the type used to visualize ethidium bromide on agarose gels). The 705 and 800 nm quantum dot emission cannot be seen by eye, but is detected by many cameras and detectors. Though we have not seen pronounced loss of fluorescence of these materials under any storage conditions that we have investigated, we have not been able to examine all storage conditions. If the Qdot® streptavidin conjugates do not appear to fluoresce under the long wave UV excitation, contact Technical Support (probestech@invitrogen.com) for assistance.

Specificity and titer of primary antibody

Make sure the antibody recognizes the intended targets and that there is sufficient primary antibody bound to the targets. This verification can be performed by ELISA based capture of the antigen of interest, or by other techniques that can be found in lab manuals such as the *Current Protocols in Immunology*.[22]

Biotinylation of antibody

Make sure your antibodies (the primary antibodies for two-layer and the secondary antibodies for three-layer detection) are effectively biotinylated. It may be necessary to independently adjust the concentration of the primary and secondary antibodies used in the assay to obtain optimal signal and minimal background.

High Background

High level of endogenous biotin

Increasing blocking times for the Endogenous Biotin Blocking Kit (Invitrogen Cat. no. E21390) steps may help reduce background due to endogenous biotin.

BSA lot variability

BSA used in the blocking buffer can vary by lot and producer. If unacceptable background is observed, re-optimization of blocking conditions may be required for best results when substituting alternate sources for BSA.

Antibody and Qdot® conjugate concentration optimization

Adjusting the level of biotinylated antibody for the staining can often be used to optimize the specific signal. High levels of biotinylated antibody maybe necessary to obtain the specific labeling, but overly high levels contribute to the nonspecific binding of the antibody to the sample. Nonspecifically bound biotinylated antibody will bind to the Qdot® streptavidin conjugate, resulting in higher background staining.

Appendix 2: Optimal Usable Filter Sets for Qdot® Conjugates

Table 2. Omega Optical filter set for Qdot® streptavidin conjugates.

Color	Optimal filter sets	Usable filter sets
525	XF301 Qdot®525 filter set (Exciter 1: 425DF45 or Exciter 2: 415WB100/ Dichroic: 475DCLP Emitter: 525WB20)	XF100-3, XF100-2, XF115-2, XF89-2
565	XF302 Qdot® 565 filter set (Exciter 1: 425DF45 or Exciter 2: 415WB100/ Dichroic: 475DCLP/ Emitter 565WB20)	XF104-2, XF105-2
585	XF303 Qdot® 585 filter set (Exciter: 1 425DF45 or Exciter 2: 415WB100/ Dichroic: 475DCLP/ Emitter: 585WB20)	XF101-2, XF137-2, XF152-2
605	XF304 Qdot® 605 filter set (Exciter 1: 425DF45 or Exciter 2: 415WB100/ Dichroic: 475DCLP/ Emitter: 605WB20)	XF108-2, XF102-2, XF103-2
655	XF305 Qdot® 655 filter set (Exciter 1: 425DF45 or Exciter 2: 415WB100/ Dichroic: 475DCLP/ Emitter: 655WB20)	XF102-2, XF40-2, XF42, XF45
705*	XF306 Qdot® 705 filter set (Exciter 1: 425DF45 or Exciter 2: 415WB100/ Dichroic: 475DCLP/ Emitter: 710AF40)	XF140-2, XF70, XF110-2, XF141-2, XF48-2
800 *	XF307 Qdot® 800 filter set (Exciter 1: 425DF45 or Exciter 2: 415WB100/ Dichroic: 475DCLP/ Emitter: 800WB80)	XF308 Qdot® 800 filter set for multiplexing (Exciter 1: 425DF45 or Exciter 2: 415WB100/ Dichroic: 475DCLP/ Emitter: 840WB80)
All colors†	XF300 Qdot® filter set (Exciter 1: 425DF45 or Exciter 2: 415WB100/ Dichroic: 475DCLP Emitters: 800WB80, 840WB80, 710AF40, 655WB20, 605WB20, 585WB20, 565WB20, and 525WB20)	XF129-2, XF130-2

*The 705 and 800 nm quantum dot emission cannot be seen by eye and must be detected with an IR-sensitive detector.
†For viewing multiple colors of Qdot® nanocrystals through microscope eyepieces.

Table 3. Semrock filter sets for Qdot® streptavidin conjugates.

Color	Optimal filter sets	Usable filter sets
525	Brightline® QD525-A Filter Sets: QD525-A-000 or QD525-A-000-ZERO (Exciter: FF01-435/40-25) (Dichroic: FF510-Di01-25 x 36) (Emitter: FF01-525/15-25)	GFP-3035B
565	--	FITC-3504B or YFP-2427A
585	--	TRITC-A
605	Brightline® QD605-A Filter Sets: QD605-A-000 or QD605-A-000-ZERO (Exciter: FF01-435/40-25) (Dichroic: FF510-Di01-25 x 36) (Emitter: FF01-605/15-25)	TRITC-A
625	Brightline® QD625-A Filter Sets: QD625-A-000 or QD625-A-000-ZERO (Exciter: FF01-435/40-25) (Dichroic: FF510-Di01-25 x 36) (Emitter: FF01-625/15-25)	Texas Red® (4040B)
655	Brightline® QD655-A Filter Sets: QD655-A-000 or QD655-A-000-ZERO (Exciter: FF01-435/40-25) (Dichroic: FF510-Di01-25 x 36) (Emitter: FF01-655/15-25)	Texas Red® (4040B)
705*	--	Cy5-4040A or Cy5.5-A
800*	--	Cy7-A
LP multi†	QDLP-A Filter Set: QDLP-A-000 (Exciter: FF01-435/40-25) (Dichroic: FF510-Di01-25 x 36) (Emitter: FF01-500/LP-25)	CFW-LP01-CLINICAL

*The 705 and 800 nm quantum dot emission cannot be seen by eye and must be detected with an IR-sensitive detector.
†For viewing multiple colors of Qdot® nanocrystals through microscope eyepieces.

Table 4. Chroma Technology filter sets for Qdot® streptavidin conjugates.

Color	Optimal filter sets	Usable filter sets
525	Qdot® 525 filter set (20 nm EM; 32006) (460SPUV/475DCXRU/D525/20nm) Qdot® 525 filter set (40 nm EM; 32010) (460SPUV/475DCXRU/D525/40nm)	FITC/RSGFP/Bodipy®/Fluo-3/DiO (41001), FITC/RSGFP Longpass (40012), BFP to GFP FRET (31032), BFP to GFP FRET wide excitation (31034), GFP wide blue excitation (31054)
565	Qdot® 565 filter set (20 nm EM; 32005) (460SPUV/475DCXRU/D565/20nm) Qdot® 565 filter set (40 nm EM; 32009) (460SPUV/475DCXRU/D565/40nm)	Eosin (41011), Cascade Yellow™ (31038), JP2(YGFP with EGFP-31040, Auramine (31015)
585	Qdot® 585 filter set (20 nm EM; 32004) (460SPUV/475DCXRU/D585/20nm) Qdot® 585 filter set (40 nm EM; 32008) (460SPUV/475DCXRU/D585/40nm)	R-PE (41003), Rhodamine LP (41032, FITC/PI (41016)
605	Qdot® 605 filter set (20 nm EM; 32003) (460SPUV/475DCXRU/D605/20nm) Qdot® 605 filter set (40 nm EM; 32007) (460SPUV/475DCXRU/D605/40nm)	Cy3 narrow excitation (41007a), Texas Red®/Cy3.5 (31004), TRITC (41002, 41002a, 41002b), Ethidium Bromide (41006)
655	Qdot® 655 filter set (20 nm EM; 32011) (460SPUV/475DCXRU/D655/20nm) Qdot® 655 filter set (40 nm EM; 32012) (460SPUV/475DCXRU/D655/40nm)	Texas Red® (41004), Propidium Iodide (41005), Fura Red™ (31012), Chlorophyll (31017), Allophycocyanin (31006)
705*	Qdot® 705 filter set (20 nm EM; 32014) (460SPUV/475DCXRU/D705/20nm) Qdot® 705 filter set (40 nm EM; 32015) (460SPUV/475DCXRU/D705/40nm)	Cy5 Longpass (41024), Cy5 (41008), Cy5 narrow excitation (41033), Cy5.5 (41023), Alexa Fluor® 680 (41042), Cy5.5 (red-shifted; 41022)
800*	Qdot® 800 filter set (30 nm EM; 32020) (460SPUV/475DCXRU/D800/30nm) Qdot® 800 filter set (50 nm EM; 32021) (460SPUV/475DCXRU/D800/50nm)	Cy7 (41009), Li-Cor for IRDye 800 (41037), Cy7 (SP106)
All colors†	Qdot® Multiple Emission Set (71014) (460SPUV, 475DCXRU, D525/20nm, D605/20nm, D565/20nm, D585/20nm)	UV (11000V2), Blue/Violet (11003V2), UV/Violet (11011V2)

*The 705 and 800 nm quantum dot emission cannot be seen by eye and must be detected with an IR-sensitive detector.
†For viewing multiple colors of Qdot® nanocrystals through microscope eyepieces.

Appendix 3: Extinction Coefficients

Table 5. Extinction coefficients of Qdot® streptavidin conjugates at common excitation wavelengths.

Product	350 nm, in $cm^{-1}M^{-1}$	405 nm, in $cm^{-1}M^{-1}$	488 nm, in $cm^{-1}M^{-1}$	532 nm, in $cm^{-1}M^{-1}$
Qdot® 525 nanocrystals	710,000	360,000	130,000	Not applicable
Qdot® 565 nanocrystals	1,900,000	1,100,000	290,000	139,000
Qdot® 585 nanocrystals	3,500,000	2,200,000	530,000	305,000
Qdot® 605 nanocrystals	4,400,000	2,800,000	1,100,000	580,000
Qdot® 625 nanocrystals	14,700,000	9,900,000	2,700,000	870,000
Qdot® 655 nanocrystals	9,100,000	5,700,000	2,900,000	2,100,000
Qdot® 705 nanocrystals	12,900,000	8,300,000	3,000,000	2,100,000
Qdot® 800 nanocrystals	12,600,000	8,000,000	3,000,000	2,000,000

Appendix 4: Bibliography

There are a number of references that describe the size-dependent properties of the semiconductor nanocrystals. These range in complexity from fairly straightforward descriptions to fairly comprehensive mathematical and physical descriptions of the optical properties. In addition, we have included some representative references that describe the core-shell structures, and the improved chemical properties that are obtained through such structures. References 8–11 describe quantum dots and FRET:

1. Sci Am 285, 66 (2001); **2.** J Phys Chem B 100, 13226 (1996); **3.** J Am Chem Soc 115, 8706 (1993); **4.** Phys Rev B 53, 16338 (1996); **5.** J Phys Chem 100, 468 (1996); **6.** J Phys Chem B. 101, 9463 (1997); **7.** J Am Chem Soc 119, 7019 (1997); **8.** Nano Lett 1, 469 (2001); **9.** J. Am. Chem. Soc 126, 301 (2004); **10.** Nat Mater 2, 630 (2003); **11.** Nat Biotechnol 21, 1387 (2003).

A number of references describe the biological properties of some quantum dots used in experiments. These papers are selected to represent some of the different classes of applications, but this list is not exhaustive. These materials are all quite different from the Qdot® conjugates that are sold by Invitrogen, and the results are not necessarily representative of results attainable with these materials:

12. Science 281, 2013 (1998); **13.** Science 281, 2016 (1998); **14.** J Am Chem Soc 124, 4586 (2002); **15.** Proc Natl Acad Sci U S A. 99, 12617 (2002); **16.** Science 298, 1759 (2002); **17.** Nat Biotechnol 21, 41 (2003); **18.** Nat Biotechnol 21, 47 (2003).

Also of interest:

19. Hermanson, GT. *Bioconjugate Techniques*, Academic Press, 1996; **20.** Lakowicz, J. *Principles of Fluorescence Spectroscopy*. Kluwer Academic Publishing, 1999; **21.** J Am Chem Soc 128, 12800 (2006); **22.** Colligan et. al. *Current Protocols in Immunology*. J. Wiley, Annually Updated, 2002.

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

Cat no.	Product Name	Unit Size
A10196	Qdot® 625 streptavidin conjugate *1 µM solution*	200 µL
Q10101MP	Qdot® 605 streptavidin conjugate *1 µM solution*	200 µL
Q10103MP	Qdot® 605 streptavidin conjugate, starter kit *1 µM solution*	50 µL
Q10111MP	Qdot® 585 streptavidin conjugate *1 µM solution*	200 µL
Q10113MP	Qdot® 585 streptavidin conjugate, starter kit *1 µM solution*	50 µL
Q10121MP	Qdot® 655 streptavidin conjugate *1 µM solution*	200 µL
Q10123MP	Qdot® 655 streptavidin conjugate, starter kit *1 µM solution*	50 µL
Q10131MP	Qdot® 565 streptavidin conjugate *1 µM solution*	200 µL
Q10133MP	Qdot® 565 streptavidin conjugate, starter kit *1 µM solution*	50 µL
Q10141MP	Qdot® 525 streptavidin conjugate *1 µM solution*	200 µL
Q10143MP	Qdot® 525 streptavidin conjugate, starter kit *1 µM solution*	50 µL
Q10151MP	Qdot® Streptavidin Sampler Kit *1 µM solutions*	1 kit
Q10161MP	Qdot® 705 streptavidin conjugate *1 µM solution*	200 µL
Q10163MP	Qdot® 705 streptavidin conjugate, starter kit *1 µM solution*	50 µL
Q10171MP	Qdot® 800 streptavidin conjugate *1 µM solution*	200 µL
Q10173MP	Qdot® 800 streptavidin conjugate, starter kit *1 µM solution*	50 µL
A1310	7-aminoactinomycin D (7-AAD)	1 mg
A21355	anti-OxPhos Complex V inhibitor protein, mouse IgG1, monoclonal 5E2 (anti-ATP synthase IP; anti-F1F0-ATPase IP) *human mitochondrial reactivity*	100 µg
E21390	Endogenous Biotin-Blocking Kit *100 assays*	1 kit
P3356	Propidium iodide *1.0 mg/mL solution in water*	10 mL
S33025	SelectFX® Nuclear Labeling Kit *DAPI, SYTOX® Green, 7-AAD, TO-PRO®-3 iodide* *for fixed cells*	1 kit
S7020	SYTOX® Green nucleic acid stain *5 mM solution in DMSO*	250 µL
T3605	TO-PRO®-3 iodide (642/661) *1 mM solution in DMSO*	1 mL
39-6400	Mouse anti-Nucleolin	100 µg
70011-044	Phosphate-Buffered Saline (PBS), pH 7.4 (10X), liquid	500 mL

Contact Information

Molecular Probes, Inc.
29851 Willow Creek Road
Eugene, OR 97402
Phone: (541) 465-8300
Fax: (541) 335-0504

Customer Service:
6:00 am to 4:30 pm (Pacific Time)
Phone: (541) 335-0338
Fax: (541) 335-0305
probesorder@invitrogen.com

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8:00 am to 4:00 pm (Pacific Time)
Phone: (541) 335-0353
Toll-Free (800) 438-2209
Fax: (541) 335-0238
probestech@invitrogen.com

Invitrogen European Headquarters
Invitrogen, Ltd.
3 Fountain Drive
Inchinnan Business Park
Paisley PA4 9RF, UK
Phone: +44 (0) 141 814 6100
Fax: +44 (0) 141 814 6260
Email: euroinfo@invitrogen.com
Technical Services: eurotech@invitrogen.com

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