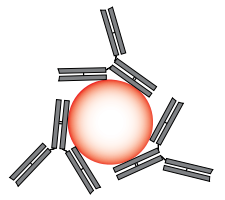
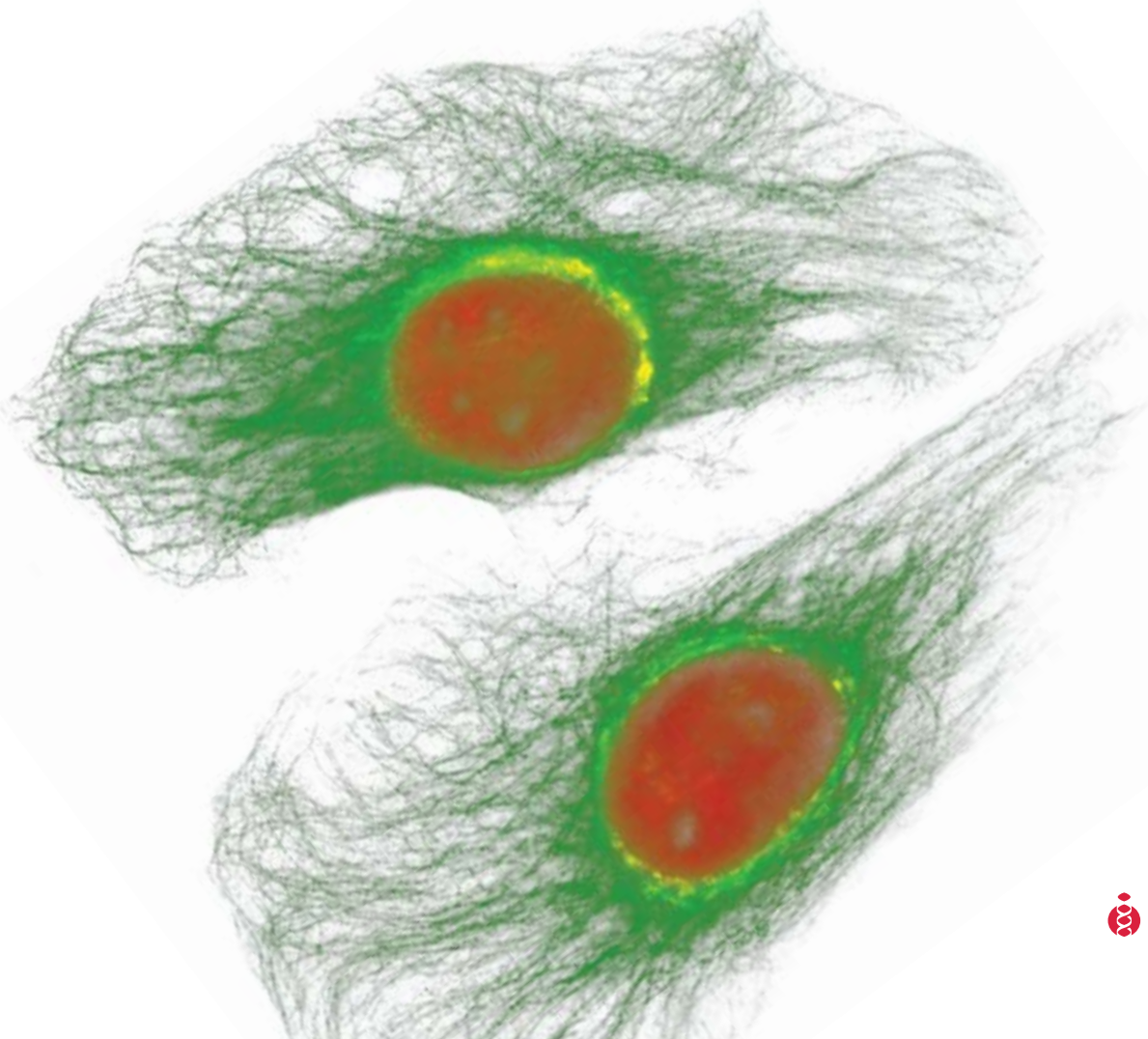


Qdot® Nanocrystals



The future of fluorescence

Qdot® nanocrystal technology



Qdot® nanocrystals: The vision of nanotechnology

Qdot® nanocrystals offer revolutionary fluorescence performance:

- Long-term photostability for live-cell imaging and dynamics studies
- Brilliant colors for simple, single-excitation multicolor analysis
- Fixability for follow-up immunofluorescence after *in vivo* studies
- Narrow, symmetrical emission spectra for low interchannel crosstalk

What are Qdot® nanocrystals?

Fundamentally, Qdot® nanocrystals are fluorophores—substances that absorb photons of light, then reemit photons at a different wavelength.¹⁻³ However, simple comparisons with organic fluorescent dyes and naturally fluorescent proteins end there. Qdot® nanocrystal conjugates combine exceptional fluorescence with full biofunctionality for a wide variety of life science applications. This combination is especially important for those applications requiring excellent photostability and multicolor detection from a single excitation source.

Anatomy of a Qdot® nanocrystal

Qdot® nanocrystals are nanometer-scale (roughly protein-sized) atom clusters comprising a core, shell, and coating (Figures 1 and 2). The core is made up of a few hundred to a few thousand atoms of a semiconductor material (often cadmium mixed with selenium or tellurium). A semiconductor shell (zinc sulfide) surrounds and stabilizes the core, improving

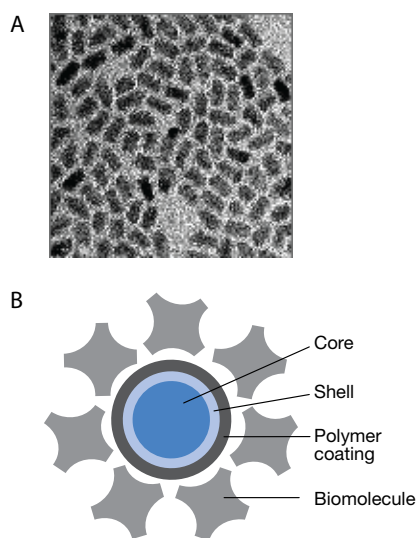


Figure 2—Structure of a Qdot® nanocrystal. A. Qdot® nanocrystals with core and shell components only are shown in this transmission electron microscope image (200,000× magnification). B. In this schematic of the overall structure of a Qdot® nanocrystal streptavidin conjugate, the layers represent the distinct structural elements and are roughly to scale.

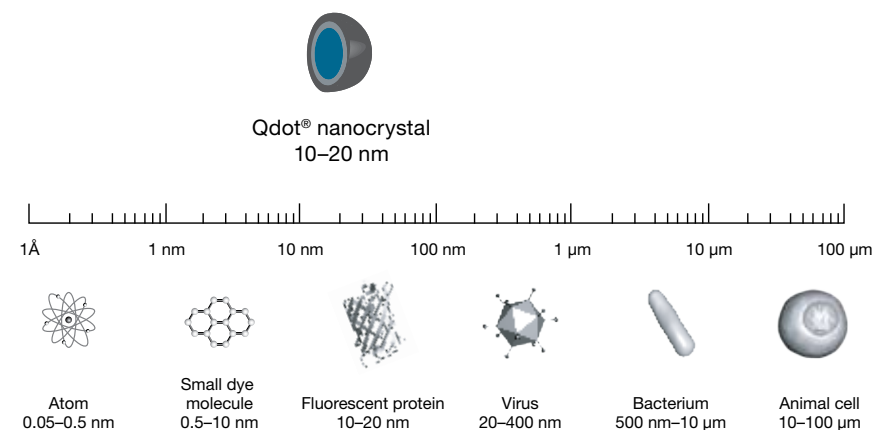


Figure 1—Relative size (hydrodynamic diameter in nm) of Qdot® nanocrystals. Qdot® nanocrystals are roughly protein-sized clusters of semiconductor material.

both the optical and physical properties of the material. An amphiphilic polymer coating then encases this core and shell, providing a water-soluble surface that can be differentially modified to create Qdot® nanocrystals that meet specific assay requirements.

For most of the Qdot® nanocrystal products, this amphiphilic inner coating is covalently modified with a functionalized polyethylene glycol (PEG) outer coating. The PEG surface has been shown to reduce nonspecific binding in flow cytometry⁴ and imaging assays, thereby improving signal-to-noise ratios and providing clearer resolution of cell populations and cellular morphology. Qdot® primary and secondary antibody conjugates (Figure 3), Qdot® streptavidin conjugates, Qtracker® nontargeted quantum dots, and Qdot® ITK™ amino (PEG) quantum dots, as well as the reactive nanocrystals provided in the Qdot® Antibody Conjugation Kit, all utilize this PEG chemistry.

Fluorescence of Qdot® nanocrystals

Qdot® nanocrystals are extremely efficient materials for generating fluorescence (Table 1). Their intrinsic brightness is often many times that observed for traditional organic fluorophores, and their photostability is many orders of magnitude greater. These extraordinary fluorescence properties can be attributed to the unique fluorescence mechanism of semiconductor materials. Unlike organic fluorophores, Qdot® nanocrystals fluoresce through the formation of excitons, or Coulomb-correlated electron–hole pairs, upon absorption of a photon of light. Compared with the excited state of a fluorophore, this exciton typically exhibits a much longer lifetime (up to ~200 nanoseconds), a property that can be advantageous in certain types of time-gated detection studies.⁵

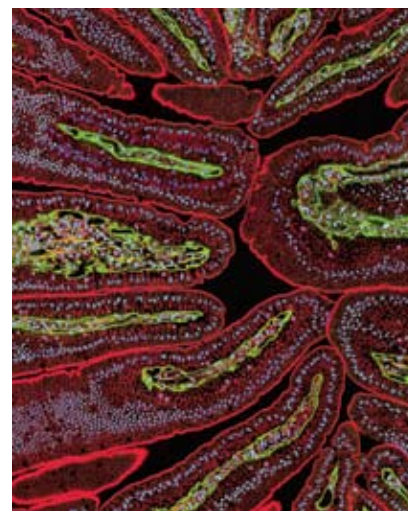


Figure 3—Multicolor immunofluorescence imaging with Qdot® secondary antibody conjugates. Actin in a mouse intestine section was detected with mouse anti-actin antibody and Qdot® 655 goat F(ab')₂ anti-mouse IgG antibody (red), laminin was detected with rabbit anti-laminin antibody and Qdot® 525 goat F(ab')₂ anti-rabbit IgG antibody (green), and nuclei were stained with Hoechst 33342 (blue). Image contributed by Thomas Deerinck and Mark Ellisman, The National Center for Microscopy and Imaging Research, San Diego, California, USA.

Table 1—Extinction coefficients of Qdot® streptavidin conjugates at common excitation wavelengths.

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

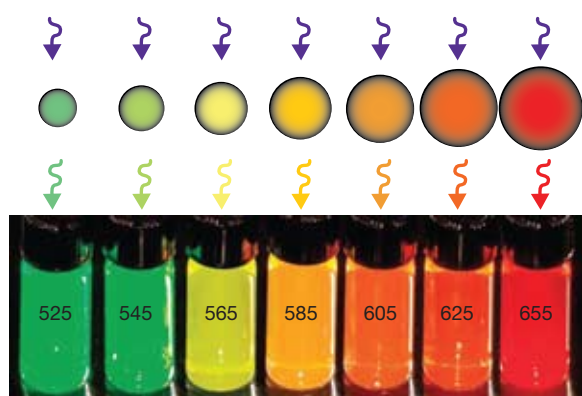


Figure 4—Tuneability of Qdot® nanocrystals. Seven different nanocrystal solutions are shown excited with the same long-wavelength UV lamp; the size of the nanocrystal at the angstrom scale determines the color.

Tuneability of Qdot® nanocrystals

In addition to these distinctive structural and fluorescence properties, Qdot® nanocrystals show a direct, predictable relationship between their physical size and the energy of the exciton (and therefore, the wavelength of emitted fluorescence) (Figure 4). This property, referred to as “tuneability”, has allowed us to develop a series of Qdot® nanocrystals that have a common excitation profile but different fluorescent emission maxima. The tuneability of Qdot® nanocrystals is being widely exploited in the development of multicolor nanocrystal-based assays.

What can Qdot® nanocrystals do?

Brightness coupled with photostability for long-term studies

The remarkable photostability of Qdot® nanocrystals enables long-term imaging experiments under conditions that would lead to the photoinduced deterioration of other types of fluorophores.^{3,6} Howarth and colleagues have achieved real-time imaging of the complex formed between a single ligand-labeled nanocrystal and its target receptor on live neurons,⁷ an approach whose success these authors attribute in part to the brightness inherent in the nanocrystal particles. Furthermore, cells and tissues labeled with Qdot® nanocrystals can be archived permanently and reanalyzed with the same level of sensitivity as achieved in the initial assay.

Nanocrystal emission series for multicolor analysis

With their broad excitation and narrow, symmetric emission properties (Figure 5), the Qdot® nanocrystals require only a single excitation source (typically <450 nm), facilitating multiplex analysis of multiple targets or events in a single sample. For example, Chattopadhyay and coworkers used eight different Qdot® nanocrystals in combination with organic fluorophores to achieve 17-color immunophenotyping by flow cytometry.⁴ Proper filter selection is critical, however, for resolving individual fluorescent signals arising

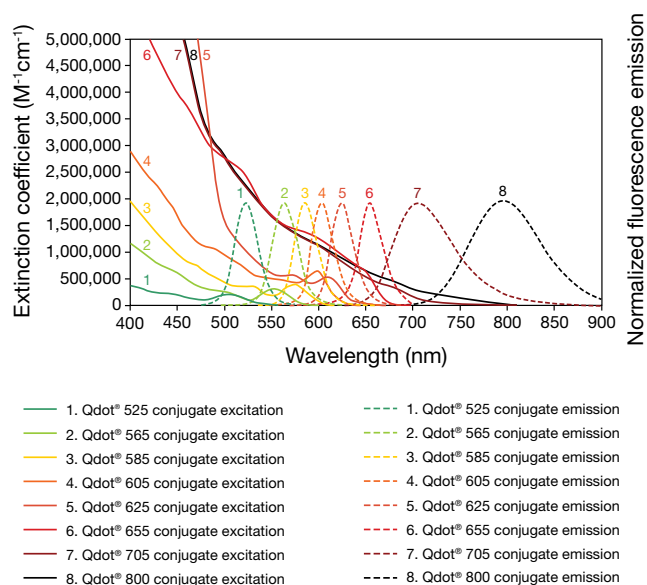


Figure 5—Absorption and emission profiles of Qdot® nanocrystals. Qdot® nanocrystals are characterized by broad absorption spectra and narrow, symmetrical, and discrete emission profiles. Note that the absorption profiles shown here for these Qdot® nanocrystals are identical to their excitation profiles.

from different Qdot® nanocrystals; recommended filter sets are available from the major filter manufacturers (Table 2). Because nanocrystals are particle-based fluorophores, they have intrinsic electron and X-ray contrast, delivering powerful multimodality for correlative light and electron microscopy and for imaging studies that utilize both fluorescence and X-ray or computerized tomography (CT).

Diverse surface chemistries for customization

The surface chemistry dictates many of the important properties of the Qdot® nanocrystals in biological context. For that reason, Qdot® nanocrystals are available with a choice of surface reactivities, from the nonreactive Qtracker® nontargeted quantum dots for *in vivo* imaging, to the amine- and carboxyl-derivatized Qdot® Innovator’s Tool Kit (ITK™) quantum dots. We also prepare Qdot® ITK™ nanocrystals labeled with streptavidin for creating brightly fluorescent noncovalent conjugates with biotinylated molecules, as well as Qdot® ITK™ organic quantum dots for applications requiring organic solvents. The diverse surface functionalities available in the Qdot® product line offer researchers a multitude of choices for creating custom nanocrystal conjugates.

Qdot® 625 nanocrystals and conjugates

A better, brighter red nanocrystal for immunostaining, flow cytometry, and more

The first of our next-generation quantum dots, the Qdot® 625 nanocrystals, are now available from Invitrogen. This new color complements our series of visible and near-infrared colors of Qdot® nanocrystals (Figure 6), making the possibility of true eight-color multispectral analyses a reality for many researchers. Qdot® 625 nanocrystals exhibit the same exceptional photostability and narrow emission profile as the other Qdot® nanocrystal colors but without

Table 2—Spectral properties and recommended filters for Qdot® nanocrystals.

Qdot® nanocrystal	Excitation wavelength (nm)*	Peak emission wavelength (nm)	Recommended filter
Qdot® 565	405	565	575/26
Qdot® 605	405	605	605/20
Qdot® 625	405	625	625/20
Qdot® 655	405†	655	655/20
Qdot® 705	405†	705	720/20
Qdot® 800	405†	800	787/42

*Qdot® nanocrystals excite optimally in the UV and 405 nm range, but can also be excited with 488 nm lasers.
† Can also be excited with 633 nm lasers.

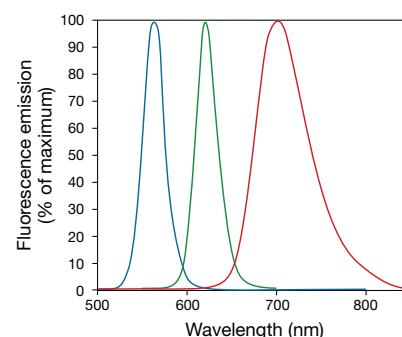


Figure 6—Fluorescence emission spectra of Qdot® 565, Qdot® 625, and Qdot® 705 streptavidin conjugates. In the context of multiplex detection with Qdot® 565 and Qdot® 705 conjugates, the relative position of the Qdot® 625 emission envelope is superior in terms of signal crosstalk to Qdot® 605 or Qdot® 655 conjugates. In such applications, all Qdot® conjugates can be efficiently excited at a single wavelength in the near-UV/violet region (e.g., 405 nm).

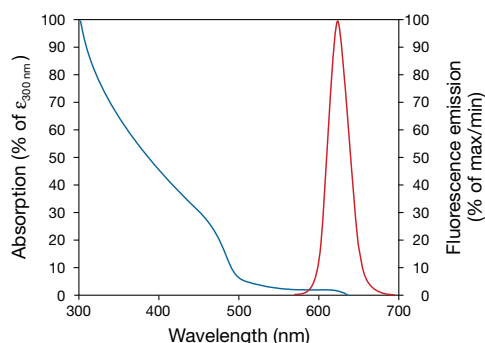


Figure 7—Absorption (blue) and emission (red) spectra of Qdot® 625 streptavidin conjugate.

Table 3—Extinction coefficients of Qdot® streptavidin conjugates at common excitation wavelengths, in $M^{-1} cm^{-1}$. For comparison, the extinction coefficient of fluorescein with 488 nm excitation is $\sim 80,000 M^{-1} cm^{-1}$.

Product	405 nm	488 nm
Qdot® 525 nanocrystals	360,000	130,000
Qdot® 565 nanocrystals	1,100,000	290,000
Qdot® 585 nanocrystals	2,200,000	530,000
Qdot® 605 nanocrystals	2,800,000	1,100,000
Qdot® 625 nanocrystals	9,900,000	2,700,000
Qdot® 655 nanocrystals	5,700,000	2,900,000

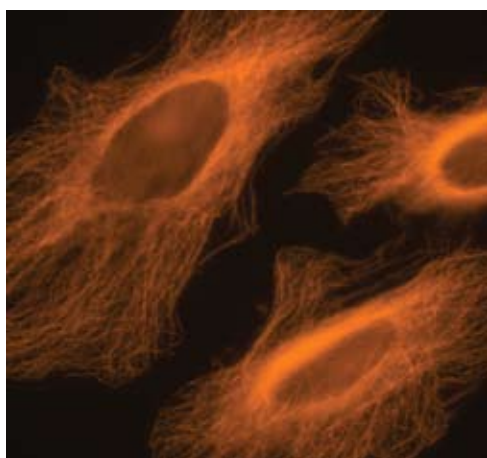


Figure 8—Qdot® 625 goat anti-mouse conjugate immunolabeling of microtubules in HeLa cells. Fixed and permeabilized HeLa cells were labeled with mouse anti- α -tubulin primary antibody and 20 nM Qdot® 625 goat anti-mouse IgG.

the “red shoulder” often observed with traditional dyes (Figure 7). As evidenced by the extinction coefficients using 405 nm and 488 nm excitation, Qdot® 625 nanocrystals are the brightest of the visible-range quantum dots (Table 3).

Qdot® 625 nanocrystals for a multitude of applications

Our Qdot® 625 streptavidin and goat anti-mouse IgG and anti-rabbit IgG secondary antibody conjugates provide a high level of resolution and brightness for immunostaining of cells grown in culture (Figure 8). For those who wish to conjugate their own antibodies to these ultrabright nanocrystals, the Qdot® 625 Antibody Conjugation Kit is available. For optimal detection of the Qdot® 625 nanocrystals, we recommend the BrightLine® QD625-A Filter Set (visit www.semrock.com). The Qtracker® 625 Cell Labeling Kit provides researchers with the means to nonspecifically load Qdot® nanocrystals into live cells for long-term cell tracking. Invitrogen has tested this new material in both CHO and HeLa cells, and from experience with our other Qdot® colors used for cell labeling, many other cell types should also be compatible with the 625 dots. In addition, Qdot® 625 conjugates are compatible with flow cytometry and exhibit their best utility when used in instruments equipped with violet lasers (Figure 9).

Because the electron-dense Qdot® nanocrystals have uniform size distribution and shape, some researchers have successfully correlated fluorescence data with electron microscopy data to resolve the spatial location of targets immunostained with Qdot® conjugates.⁸ This utility is further exploitable because some of the Qdot® nanocrystals themselves have distinguishable shapes (Qdot® 605 and Qdot® 655 are rod shaped, whereas the rest of the Qdot® nanocrystals, including Qdot® 625, are spherical (Figure 10)), making resolution of dual-stained samples at the near-molecular level a possibility.

The compatibility of Qdot® 625 nanocrystals with existing Qdot® colors makes it possible to perform multispectral analysis with all eight Qdot® nanocrystal colors using a single excitation source. The exceptional brightness exhibited by Qdot® 625 nanocrystals offers a distinct advantage when detecting low-abundance targets. These materials may also prove useful for new areas of bioresearch that involve investigating the spatial location and tracking of single molecules in live cells. Learn more about the advantages of Qdot® nanocrystals at www.invitrogen.com/qdots.

Ordering information

Product	Quantity	Cat. no.
Qdot® 625 Streptavidin Conjugate, 1 µM solution	200 µl	A10196
Qdot® 625 Goat F(ab') ₂ Anti-Mouse IgG Conjugate (H+L), 1 µM solution, highly cross-adsorbed	100 µl	A10195
Qdot® 625 Goat F(ab') ₂ Anti-Rabbit IgG Conjugate (H+L), 1 µM solution, highly cross-adsorbed	100 µl	A10194
Qtracker® 625 Cell Labeling Kit	1 kit	A10198
Qdot® 625 ITK™ Carboxyl Quantum Dots, 8 µM solution	250 µl	A10200
Qdot® 625 Antibody Conjugation Kit	1 kit	A10197

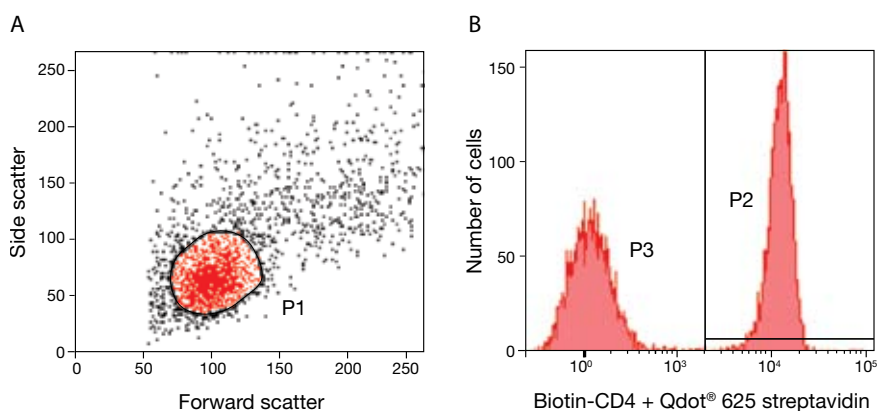


Figure 9—Qdot® 625 streptavidin conjugate for the characterization of human lymphocytes using flow cytometry. Human lymphocytes were first labeled with a mouse anti-human CD4 biotin conjugate. After washing with 1% BSA/PBS, the cells were then incubated with Qdot® 625 streptavidin conjugate for 15 minutes at room temperature. Cells were washed again with 1% BSA/PBS and analyzed on an LSR II flow cytometer (BD Biosciences). When gated on lymphocytes (P1 in **A**), the histogram (**B**) shows very good peak separation between the CD4-positive (P2) and -negative (P3) populations. The PMT setting was 500 V, and a 600 nm longpass dichroic mirror and a 630/22 nm bandpass filter were used.

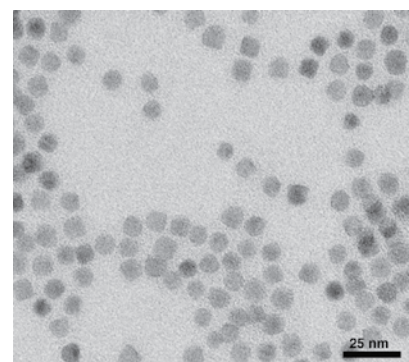


Figure 10—Qdot® 625 streptavidin transmission electron microscopy (TEM) images. Courtesy of Mark Ellisman, National Center for Microscopy and Imaging Research, University of California, San Diego, San Diego, CA.

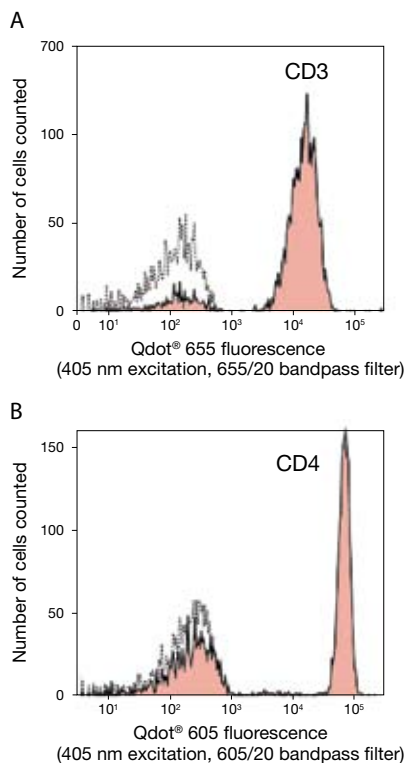


Figure 11—Antigen detection in human peripheral blood lymphocytes. Cells were incubated with mouse anti-human CD3 antibody labeled with Qdot® 655 (A) or mouse anti-human CD4 antibody labeled with Qdot® 605 (B) and subjected to flow cytometric analysis using LSR II flow cytometers and FACSDiva™ software (BD Biosciences) or FlowJo® software (Tree Star, Inc.). The gray overlay peaks represent a sample of unstained cells.

Qdot® nanocrystal–conjugated primary antibodies

- Flexibility—excited by 405 nm or 488 nm light, allowing researchers to maximize the use of violet or blue lasers
- Compatibility—can be used in combination with existing organic dyes to increase the number of detectable parameters
- Stability—do not degrade over time like tandem conjugates, giving greater reproducibility
- Minimal single-laser compensation—narrow emission spectra require minimal compensation when using a single excitation source

To match the ever-expanding detection capabilities available in new instrumentation, Molecular Probes® Qdot® nanocrystals offer an exciting new array of fluorescent labels for use in flow cytometry (Figure 11). Qdot® nanocrystals can be excited with UV or violet light sources, as well as with longer-wavelength light sources, and exhibit long Stokes shifts and relatively narrow emission peaks. The result is greater flexibility and precision in designing multicolor flow cytometry panels.

As the exclusive provider of Qdot® nanocrystal technology for life science research, Invitrogen offers a full range of tools, from our new primary antibody conjugates to secondary detection reagents, to maximize the use of your flow cytometer by combining Qdot® nanocrystal technology with existing organic fluorophores. For more information on this new technology, including a complete list of Qdot® conjugates available for secondary detection, please visit www.invitrogen.com/qdotinflow.

Ordering information

Description	Clone	Isotype	Qdot® 565	Qdot® 605	Qdot® 625	Qdot® 655	Qdot® 705	Qdot® 800
Human CD3	UCHT1	Mouse IgG2a		Q10054				
Human CD3	S4.1	Mouse IgG2a				Q10012		
Human CD4	S3.5	Mouse IgG2a		Q10008		Q10007	Q10060	
Human CD8	3B5	Mouse IgG2a		Q10009		Q10055	Q10059	
Human CD14	Tük4	Mouse IgG2a		Q10013		Q10056		Q10064
Human CD27	CLB-27/1	Mouse IgG2a		Q10065		Q10066		
Human CD38	HIT2	Mouse IgG1		Q10053		Q10057		
Human CD45	HI30	Mouse IgG1		Q10051			Q10062	
Human CD45RA	MEM-56	Mouse IgG2b		Q10047		Q10069		
Mouse IgG1	NA	Mouse IgG1		Q10073				
Mouse IgG2a	NA	Mouse IgG2a		Q10014		Q10015	Q10076	Q10075
Mouse IgG2b	NA	Mouse IgG2b		Q10074				
Streptavidin	NA	NA	Q10131MP	Q10101MP	A10196	Q10121MP	Q10161MP	Q10171MP

All antibodies listed in this table are for Research Use Only (RUO). All antibodies and isotype controls listed are available in 0.1 ml, 100-test size (minimum). Streptavidin conjugates are available in 200 µl or 500 µl (Q10161MP) sizes. NA = Not applicable.

Qdot® nanocrystal products

Qdot® secondary antibody and streptavidin conjugates

Detecting low-abundance antigens with even the best conventional dye conjugates can be a challenge when photobleaching restricts your ability to effectively observe and record staining. Although smaller in overall size and therefore better at penetrating some tissues, fluorescent dye conjugates are typically limited in their single-excitation multicolor applications by their small Stokes shift (Figure 12). The exceptional photostability of Qdot® secondary antibody and streptavidin conjugates, as well as their expansive multiplexing capabilities, can provide substantial benefits for antigen detection by fluorescence microscopy, flow cytometry, western blot analysis, or microtiter plate-based assays.

Qdot® secondary antibody conjugates combine the spectral characteristics of Qdot® nanocrystals with the selective binding of the F(ab')₂ fragment from affinity-purified, highly cross-adsorbed secondary antibodies, enabling multicolor analysis and long-term

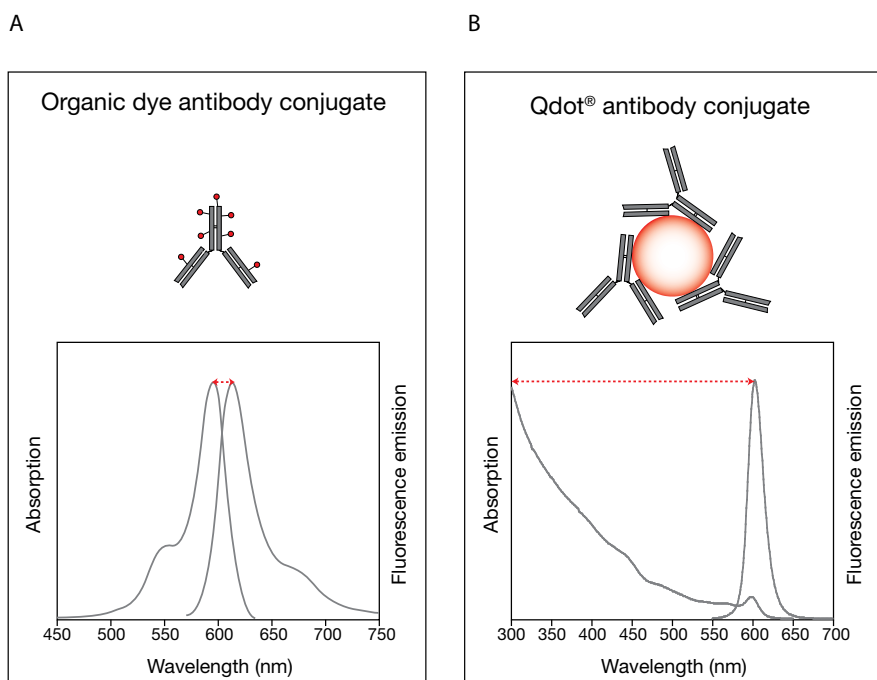


Figure 12—Comparison of organic fluorophore-labeled antibody conjugates and Qdot® nanocrystal-labeled antibody conjugates. A. The fluorescence spectra of a typical organic fluorophore (Texas Red® dye) are shown below a schematic of an organic fluorophore-labeled IgG antibody with a typical number of dyes per IgG. B. The fluorescence spectra of the Qdot® 605 nanocrystal are shown below a schematic of a Qdot® nanocrystal-labeled IgG antibody with a typical number of antibody molecules per nanocrystal. Both antibody conjugates are drawn roughly to scale, and antibody conjugates are available containing whole antibodies, F(ab')₂ fragments, or Fab fragments. Organic fluorophore-labeled antibodies are smaller in size, which may be important in some applications where accessibility of the antigen is an issue. Qdot® nanocrystal-labeled antibodies show good separation between excitation and emission wavelengths (Stokes shift shown in red) and are available with different fluorescent emission maxima for single-excitation multicolor analyses.

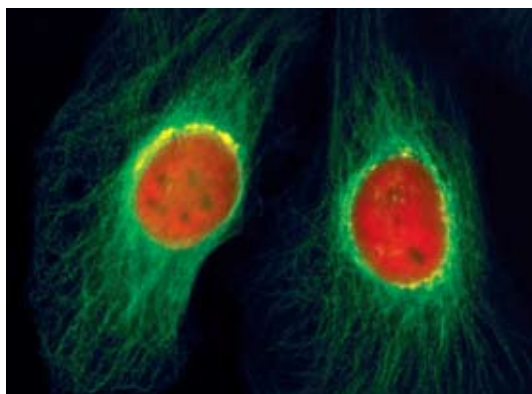


Figure 13—Multicolor immunofluorescence imaging with Qdot® secondary detection conjugates. Tubulin fibers in fixed HeLa cells were labeled with rat anti- α -tubulin antibody, biotinylated goat anti-rat IgG antibody, and Qdot® 525 streptavidin (green); Golgi bodies were labeled with rabbit anti-giantin antibody and Qdot® 585 goat F(ab')₂ anti-rabbit IgG antibody (yellow); and nuclei were labeled with mouse anti-nucleosome antibody and Qdot® 655 goat F(ab')₂ anti-mouse IgG antibody (red).

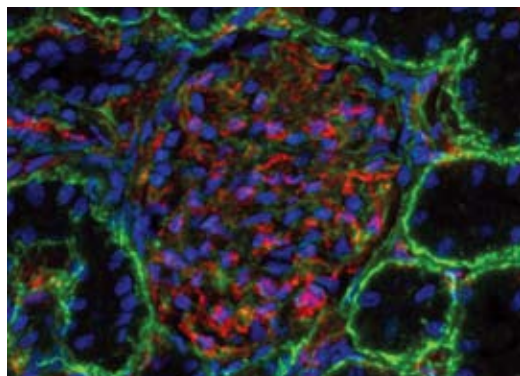


Figure 14—Multicolor immunofluorescence imaging with Qdot® secondary antibody conjugates. Laminin in a mouse kidney section was labeled with a rabbit anti-laminin primary antibody and visualized using green-fluorescent Qdot® 565 F(ab')₂ anti-rabbit IgG secondary antibody. PECAM-1 (platelet/endothelial cell adhesion molecule-1, CD31) was labeled with a rat anti-PECAM-1 primary antibody and visualized using red-fluorescent Qdot® 655 F(ab')₂ anti-rat IgG secondary antibody. Nuclei were counterstained with blue-fluorescent Hoechst 33342. Image contributed by Stuart Shand, Center for Biologic Imaging, University of Pittsburgh.

sample stability in a wide range of immunochemical applications (Figures 13 and 14). Likewise, Qdot® streptavidin conjugates have proven extremely useful for visualizing biotinylated probes in fluorescence microscopy (Figures 13 and 15) and flow cytometry, and for preparing noncovalent conjugates with biotinylated proteins. Six of the seven Qdot® streptavidin conjugates are available together in the Qdot® Streptavidin Sampler Kit (525, 565, 585, 605, 655, and 705 nm emissions). As with all of the Qdot® protein conjugates, both the Qdot® antibody and Qdot® streptavidin conjugates utilize the PEG linker chemistry to ensure high-quality staining with low background levels in standard physiological buffers (pH 6–9) in a wide range of salt concentrations.

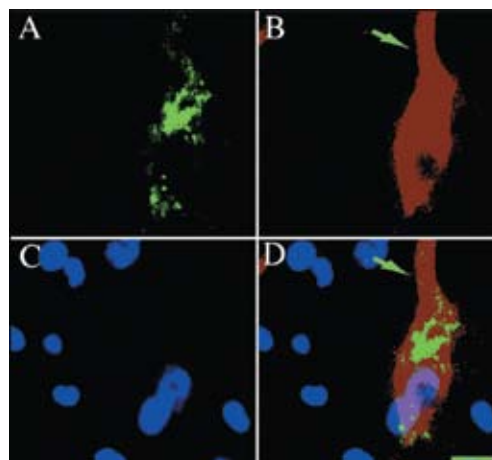


Figure 15—Multicolor immunofluorescence imaging with Qdot® streptavidin conjugates. Multiplex labeling of mRNA and protein in mouse brain was performed using Qdot® 525 and Qdot® 605 streptavidin conjugates. After *in situ* hybridization, the same tissue sections were then processed for immunohistochemistry. **A.** *Vmat2* mRNA-positive neurons in substantia nigra were probed with a biotinylated oligonucleotide and detected with Qdot® 525 streptavidin conjugate. **B.** The same cell was labeled with anti-tyrosine hydroxylase (TH) antibody in conjunction with biotinylated secondary antibody and Qdot® 605 streptavidin conjugate. The *Vmat2* mRNA signal is restricted to the cytoplasm, whereas the labeling of TH is extended to the whole cell body and processes (arrow). **C.** Cell nuclei were labeled with DAPI. **D.** Overlay of all three stained images shows the different subcellular distributions of *Vmat2* mRNA and TH immunoreactivity. Scale bar = 15 μ m. Images contributed by Stuart Sealton, Mount Sinai School of Medicine, and reprinted with permission from *Nucleic Acids Res* 33:e161 (2005).

Biotin-labeled Qdot® 605 and Qdot® 655 nanocrystals containing the PEG outer coating are also available for detecting streptavidin probes and for creating noncovalent conjugates with streptavidin-labeled molecules or with other biotinylated molecules using a streptavidin bridge.

Qdot® anti-dye conjugates

In addition to these antibody and streptavidin conjugates, we offer Qdot® 565 and Qdot® 655 conjugates of goat anti-fluorescein antibody and a Qdot® 655 conjugate of rat anti-dinitrophenyl (anti-DNP) antibody. Although widely used as a fluorochrome, fluorescein is also an excellent hapten that can be recognized by anti-fluorescein antibodies, providing an alternative to the traditional biotin–avidin system in applications such as *in situ* hybridization, enzyme-linked immunosorbent assays (ELISAs), and western blot analysis. Similarly, the DNP chromophore serves as a convenient alternative to the biotin hapten in bioconjugates because it is easy to determine the degree of substitution using the dye's visible absorption. Moreover, unlike biotin, which is an endogenous ligand in mitochondria, the fluorescein and DNP haptens allow background-free staining of cells and tissues using anti-fluorescein and anti-DNP conjugates, respectively. Many primary or secondary detection reagents, such as proteins and nucleic acid probes, can be effectively linked to fluorescein or DNP and subsequently detected with the corresponding Qdot® anti-dye conjugates.

Qdot® primary antibody conjugate

Although secondary detection methods can provide considerable signal amplification, a directly labeled fluorescent primary antibody often produces lower background levels and less nonspecific binding. The Qdot® 655 conjugate of goat anti–glutathione S-transferase (anti-GST) can be used for effective detection and

localization of GST-tagged protein fusions in fluorescence microscopy, western blot analysis, or microtiter plate–based assays.

Qdot® lectin conjugate

Fluorescent conjugates of wheat germ agglutinin (WGA), a 36 kDa protein that binds to *N*-acetylglucosamine and *N*-acetylneuraminic acid (sialic acid) residues of glycoproteins and glycolipids, are commonly used for labeling cell surfaces and for measuring retrograde neuronal transport. Our Qdot® 655 conjugate of WGA provides highly sensitive labeling of these carbohydrate residues with very low nonspecific binding.

Qdot® Antibody Conjugation Kits

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 any of eight different fluorescent colors of 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 to reactive maleimide groups, which are present on the nanocrystals after SMCC activation. In addition to antibodies, other thiol-containing molecules can be coupled to Qdot® nanocrystals using these kits. Invitrogen also offers custom conjugation services for covalently attaching your antibody or other protein of interest to Qdot® nanocrystals; please email us at probescustom@invitrogen.com for more information.

Qdot® Western Blotting Kits

In many cases, Qdot® nanocrystal fluorescence technology offers significant advantages over colorimetric and chemiluminescence methods traditionally used for western blotting. The Qdot® Western Blotting Kits are simple and easy to use, provide excep-

tional sensitivity and a broad linear range of detection, and produce two-color fluorescent western blots that can be analyzed using a simple digital camera as well as most gel documentation imaging systems. Each kit includes two compatible Qdot® secondary antibody conjugates (one anti-mouse IgG antibody and one anti-rabbit IgG antibody) for use with a pair of primary antibodies (one mouse IgG and one rabbit IgG) chosen by the researcher, along with buffers and low-autofluorescence PVDF membranes that have been optimized to produce a highly sensitive multicolor western blot (Figure 16). Two Qdot® Western Blotting Kits are available, containing either Qdot® 565 and Qdot® 655 secondary antibody conjugates or Qdot® 605 and Qdot® 705 conjugates. These secondary antibody pairs have been chosen to minimize spectral overlap between the Qdot® conjugates regardless of the imaging system used. Optimized filter sets allow higher levels of multiplexing—including use of the two Qdot® Western Blotting Kits simultaneously—increasing the amount of information obtained from a single blot without any stripping and reprobing steps.

The Western Blotting Accessory Kit contains the same buffers and PVDF membranes supplied in the Qdot® Western Blotting Kits but without the Qdot® antibody conjugate pairs. This kit is designed for use with any Qdot® nanocrystal-labeled primary or secondary antibody conjugate, either purchased from Invitrogen or, for even greater flexibility, prepared using Qdot® Antibody Conjugation Kits. On western blots, the signal amplification achieved using secondary detection methods can be significant. Using an unlabeled anti-GST primary antibody in conjunction with a Qdot® secondary antibody conjugate, we have detected 4–8 pg of GST per lane on a western blot, compared with 40–70 pg per lane using a Qdot® primary antibody conjugate (data not shown).

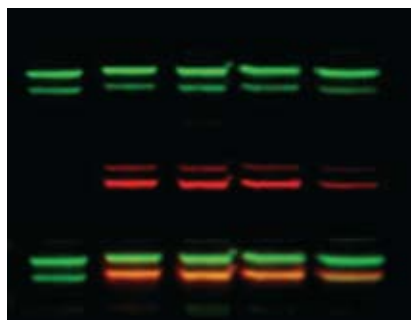


Figure 16—Multicolor western blotting using Qdot® secondary antibody conjugates. Total ERK (extracellular signal-regulated kinase) and phosphorylated ERK were labeled on blotted cell lysates with Qdot® 565 (green) and Qdot® 655 (red) nanocrystal conjugates, respectively, and detected with secondary antibody reagents.

Qtracker® Cell Labeling Kits

Qtracker® Cell Labeling Kits, which contain the reagents needed to deliver highly fluorescent Qdot® nanocrystals into the cytoplasm of live cells, provide a powerful tool for real-time cell tracking studies. To gain access to the cell cytoplasm, the Qdot® nanocrystals contain a selective targeting peptide noncovalently bound to the nanocrystal. Once

internalized by the cells, these Qdot® nanocrystals exhibit intense, photostable fluorescence that can be observed using continuous illumination without time constraints due to photobleaching or degradation. The Qdot® nanocrystals are distributed in vesicles throughout the cytoplasm (Figure 17) and are passed to daughter cells through at least six generations. Moreover, the Qdot® nanocrystals are not transferred to adjacent cells in the population, and their fluorescence is maintained in complex cellular environments and under various biological conditions, including changes in intracellular pH, temperature, and metabolic activity. In addition, experiments indicate that Qtracker® labeling does not significantly affect cell proliferation or cellular enzyme activity. These properties make the Qtracker® Cell Labeling Kits important tools for long-term studies of live cells and their functions, including adhesion, migration, motility, morphology, and transplantation.

Each Qtracker® Cell Labeling Kit contains Qdot® nanocrystals in one of seven brilliant fluorescent colors (525, 565, 585, 605, 655, 705, or 800 nm emission). These Qtracker® Cell Labeling Kits can be used together for multiplexing applications and are compatible with a variety of instrument platforms, including flow cytometry, fluorescence and confocal microscopy, fluorescence microplate readers, and high-content screening systems.

Qtracker® nontargeted quantum dots

Qtracker® nontargeted quantum dots are designed for small animal *in vivo* imaging, and especially for studying vascular structure after microinjection. These nanocrystals exhibit extremely intense fluorescence, red-shifted emission for increased tissue penetration, and a PEG surface coating specially developed to minimize nonspecific interactions and reduce any immune response by the tissue. Because the PEG surface coating does not contain reactive functional groups, the Qtracker® nontargeted quantum dots are retained in circulation longer and can be imaged for up to 3 months without additional injections. Qtracker® nontargeted quantum dots—available with 565, 655, 705, or 800 nm emission—can reveal highly detailed vascular structure at all levels of magnification (Figure 18).

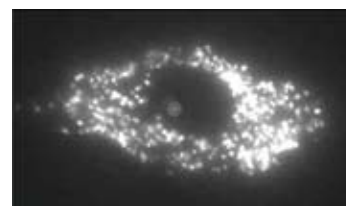


Figure 17—Distribution of Qdot® nanocrystals in cytoplasmic vesicles after labeling cells with the Qtracker® Cell Labeling Kit. HeLa cells were labeled with the Qtracker® 655 Cell Labeling Kit and then observed using a Leica TCS SP2 confocal microscope (excitation at 488 nm). This representative image shows the Qdot® nanocrystals distributed in vesicles throughout the cytoplasm.

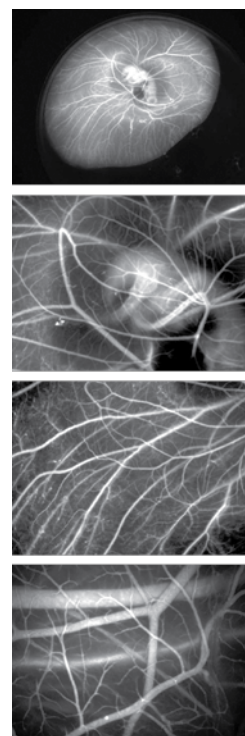


Figure 18—Chick embryo injected through the major vitelline vein with Qtracker® nontargeted quantum dots. Following a few minutes of circulation of the Qtracker® 705 nontargeted quantum dots, fluorescence images of the embryo were captured at increasing magnification using 460 nm excitation and a digital imaging system equipped with appropriate emission filters. These Qtracker® reagents revealed highly detailed vascular structure at all levels of magnification. Images contributed by Greg Fisher, Carnegie Mellon University.

Qdot® ITK™ quantum dots

Innovative science requires versatile tools. Qdot® Innovator’s Tool Kit (ITK™) quantum dots enable researchers to custom-label Qdot® nanocrystals with nearly any biomolecule of interest (Figure 19). Qdot® ITK™ quantum dots are available with three different surface chemistries—carboxyl groups, amino (PEG) groups, and organic-soluble groups—and eight different fluorescent colors (525, 545, 565, 585, 605, 655, 705, or 800 nm emission) for a multitude of labeling options.

The reactive Qdot® ITK™ quantum dots provide a remarkable platform for the development of nanocrystal-based assays, allowing researchers to experiment with any number of functional surface modifications. Qdot® ITK™ carboxyl quantum dots, which contain a carboxyl-derivatized amphiphilic coating, can be coupled to amines, hydrazines, or hydroxylamines in aqueous solution using an EDAC-mediated reaction. Kim and coworkers effectively used Qdot® ITK™ carboxyl quantum dots conjugated to a 13 amino acid peptide to characterize the preferential binding, internalization, and localization of this peptidic ligand and its payload in cancer cells.⁹ Qdot® ITK™ amino (PEG) quantum dots, which contain an amino-derivatized PEG outer coating covalently attached to the amphiphilic inner coating, efficiently react with amine-reactive groups such as isothiocyanates, succinimidyl esters, and other active esters. Qdot® ITK™ organic quantum dots have a lipophilic surface coating instead of an amphiphilic polymer coating. They are provided as a suspension in decane and are specifically designed for applications requiring organic solvents.

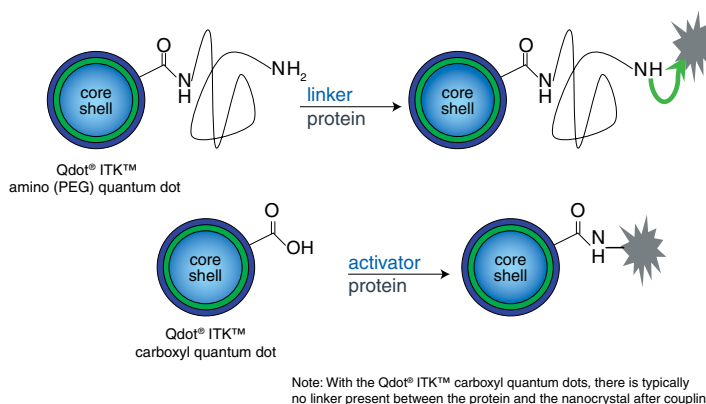


Figure 19—Coupling of Qdot® ITK™ quantum dots. Qdot® ITK™ amino (PEG) quantum dots can be coupled to biomolecules using a wide variety of standard amine-reactive crosslinking chemistries. Qdot® ITK™ carboxyl quantum dots can be coupled to biomolecules using standard EDAC (carbodiimide) activation and coupling chemistries.

We also offer Qdot® ITK™ streptavidin quantum dots, which contain streptavidin covalently attached to the inner amphiphilic coating *without* a PEG linker, for binding biotinylated probes in applications such as fluorescence resonance energy transfer (FRET). Qdot® 605 ITK™ streptavidin conjugates linked to DNA probes have been used to capture Cy®5-labeled DNA targets for the sensitive, homogeneous FRET-based detection of very low concentrations of DNA.¹⁰ In addition, the Qdot® 585 ITK™ streptavidin conjugate has been used as a FRET donor, with Cy®5 dye as FRET acceptor, to probe single-molecule structural dynamics.¹¹

The customizable surfaces of Qdot® ITK™ quantum dots should prove particularly useful in the preparation of nanocrystals with multiple surface functionalities for powerful, data-rich assays.

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Qdot® nanocrystals product selection guide

Product	Fluorescence emission maximum							
	525 nm	565 nm	585 nm	605 nm	625 nm	655 nm	705 nm	800 nm
Streptavidin and secondary antibody conjugates								
Qdot® Streptavidin Conjugate	Q10141MP	Q10131MP	Q10111MP	Q10101MP	A10196	Q10121MP	Q10161MP	Q10171MP
Qdot® Streptavidin Sampler Kit (50 µl each of 6 colors)	Q10151MP	Q10151MP	Q10151MP	Q10151MP		Q10151MP	Q10151MP	
Qdot® Biotin Conjugate				Q10301MP		Q10321MP		
Qdot® Goat F(ab') ₂ Anti-Mouse IgG Conjugate (200 µl)	Q11041MP	Q11031MP	Q11011MP	Q11001MP	A10'95	Q11021MP	Q11061MP	Q11071MP
Qdot® Goat F(ab') ₂ Anti-Mouse IgG Conjugate (100 µl) *		Q11032MP		Q11002MP		Q11022MP	Q11062MP	
Qdot® Goat F(ab') ₂ Anti-Rabbit IgG Conjugate (200 µl)	Q11441MP	Q11431MP	Q11411MP	Q11401MP	A10194	Q11421MP	Q11461MP	Q11471MP
Qdot® Goat F(ab') ₂ Anti-Rabbit IgG Conjugate (100 µl) *		Q11432MP		Q11402MP		Q11422MP	Q11462MP	
Qdot® Goat F(ab') ₂ Anti-Rat IgG Conjugate		Q11631MP		Q11601MP		Q11621MP		
Qdot® Goat F(ab') ₂ Anti-Human IgG Conjugate		Q11231MP		Q11201MP		Q11221MP		
Qdot® Goat F(ab') ₂ Anti-Chicken IgG Conjugate						Q14421MP		
Qdot® Rabbit F(ab') ₂ Anti-Goat IgG Conjugate						Q11821MP		
Anti-dye conjugates								
Qdot® Goat Anti-Fluorescein Conjugate		Q15431MP				Q15421MP		
Qdot® Rat Anti-Dinitrophenyl (DNP) Conjugate						Q17421MP		
Primary antibody conjugate								
Qdot® Goat Anti-Glutathione S-Transferase (GST) Conjugate						Q14621MP		
Lectin conjugate								
Qdot® Wheat Germ Agglutinin						Q12021MP		
Antibody conjugation kits and western blotting kits								
Qdot® Antibody Conjugation Kit	Q22041MP	Q22031MP	Q22011MP	Q22001MP	A10197	Q22021MP	Q22061MP	Q22071MP
Qdot® Western Blotting Kits (with two Qdot® secondary antibody conjugates per kit) **		Q24011MP (anti-mouse IgG)		Q24021MP (anti-mouse IgG)		Q24011MP (anti-rabbit IgG)	Q24021MP (anti-rabbit IgG)	
Nanocrystals for cell, tissue, and small animal <i>in vivo</i> labeling								
Qtracker® Cell Labeling Kit	Q25041MP	Q25031MP	Q25011MP	Q25001MP	A10198	Q25021MP	Q25061MP	Q25071MP
Qtracker® Nontargeted Quantum Dots		Q21031MP				Q21021MP	Q21061MP	Q21071MP
Nanocrystals for customizing surface properties								
Qdot® ITK™ Carboxyl Quantum Dots ***	Q21341MP	Q21331MP	Q21311MP	Q21301MP	A10200	Q21321MP	Q21361MP	Q21371MP
Qdot® ITK™ Amino (PEG) Quantum Dots ***	Q21541MP	Q21531MP	Q21511MP	Q21501MP		Q21521MP	Q21561MP	Q21571MP
Qdot® ITK™ Organic Quantum Dots ***		Q21731MP	Q21711MP	Q21701MP		Q21721MP	Q21761MP	Q21771MP
Qdot® ITK™ Streptavidin Conjugate ***	Q10041MP	Q10031MP	Q10011MP	Q10001MP		Q10021MP	Q10061MP	Q10071MP

* Specifically sized for 10 mini western blots. ** The Qdot® Western Blotting Accessory Kit (Q24001MP) contains the same buffers and PVDF membranes supplied in the Qdot® Western Blotting Kits but without the Qdot® antibody conjugates. *** These Qdot® ITK™ products are also available with 545 nm emission: Qdot® 545 ITK™ carboxyl quantum dots, Q21391MP; Qdot® 545 ITK™ amino (PEG) quantum dots, Q21591MP; Qdot® 545 ITK™ organic quantum dots, Q21791MP; Qdot® 545 ITK™ streptavidin conjugate, Q10091MP.

For current prices and more information, visit probes.invitrogen.com/products/qdot.

For inquiries about custom or bulk Qdot® nanocrystals, please contact us by email at probescustom@invitrogen.com.

