invitrogen™

Qdot® Antibody Conjugation Kit
Supplemental Information Guide

Quantum Dot
invitrogen nanocrystal technologies

Molecular Probes™
invitrogen detection technologies

PN 90-0078a, Rev 1

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Qdot® Antibody Conjugation Kit Supplemental Information Guide

Frequently Asked Questions

The properties of Qdot® nanocrystal conjugates are different than 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.

General:

1. Are Qdot® nanocrystals toxic?

   We have not investigated the toxicity of Qdot® nanocrystals. The materials provided have a total solution which is ~2 mM total Cd concentration (in the form of a CdSe core). The CdSe core is encapsulated in a shell of ZnS and the polymer shell, which may prevent dissolution 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 investigating the toxicity of the materials to humans, to animals, or to cells in culture. We recommend the use of gloves, safety glasses and lab coats with all laboratory procedures (See Appendix 5 for specific information on kit components).

2. How should I dispose of the Qdot® nanocrystal conjugate?

   The Qdot® nanocrystal conjugate contains cadmium and selenium in an inorganic crystalline form. Invitrogen can only advise that you 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.

3. Do Qdot® nanocrystals undergo FRET, or quench when they are in close proximity?

   We have not systematically investigated the energy transfer properties of Qdot® nanocrystals, though Qdot® nanocrystals may have useful properties as both energy transfer donors and acceptors. We have investigated the fluorescence of Qdot® 605 streptavidin conjugates coupled to one 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.

Conjugation and Purification:

4. I know that SMCC is moisture sensitive. How can I be sure that the solution of this compound provided with the kit is active?

   The 10 mM stock solution of SMCC in DMSO provided with the kit is prepared using high quality anhydrous DMSO. This process is carried out in a dry nitrogen atmosphere. We have studied the stability of the SMCC solution thus prepared and have found that its activity remains unchanged for a period of at least three months when stored unopened at 40 C. Once opened, hydration begins and any remaining solution should be disposed of as DMSO is hygroscopic.

5. I only have 100 µg of antibody. How can I conjugate it using the conjugation kit?

   If smaller amounts of antibody are to be conjugated, the amount of Qdot® nanocrystals in the conjugation reaction should be reduced to keep the stoichiometric ratio constant. The concentration of the final product will be correspondingly lower.

6. I want to conjugate a larger amount (600 µg) of antibody. How can I conjugate it using the conjugation kit?

   If larger amounts of antibody are to be conjugated, the amount of Qdot® nanocrystals in the conjugation reaction should be increased to keep the stoichiometric ratio constant. It may be necessary to divide the concentrated, unpurified conjugate and purify over two columns.

7. Can the ratio of antibody to Qdot® nanocrystal be varied?

   As described in the protocol, the antibody to Qdot® nanocrystal ratio is 4:1 for the 655 nm kit and 3.3:1 for the 565 nm kit. Further reduction may result in under-conjugation of the Qdot® nanocrystals.

8. I am concerned that the binding affinity of my antibody may be adversely affected by the DTT treatment. Are there any alternatives?

   The chemical process used in this kit has been successfully used by many researchers for various antibody conjugations for many years. Reduction of the disulfide bonds of intact IgG molecules has been found to have little or no effect on their affinities. However, there are alternative ways to introduce thiol groups into the antibody molecule. One of these is the reaction of the antibody with Traut's reagent. Another requires treatment with the NHS ester of S-acetyl thiolacetic acid (SATA), followed by reaction with hydroxylamine. We have successfully tested both of these and they are fully compatible with our kit. However, at this point we cannot provide any recommendations regarding specific reaction conditions.

9. Can I conjugate whole antibodies, F(ab')2, and Fab fragments using your kit?

   We have successfully conjugated such fragments of IgG class antibodies using the chemistry employed in the kit.

10. Will the dye-labeled marker used for the antibody elution off the NAP™-5 column interfere with the reaction between my antibody and the Qdot® nanocrystals? Will it be purified from my final conjugate?

    The dye-labeled marker will not interfere in the reaction. It will be purified from the final conjugate.

11. How much of the starting antibody is consumed in the reaction?

    We estimate this number to be 60-70%.

12. I collected less than the specified volume of activated dots/reduced antibody off the NAP-5 column. Is this ok?

    The volumes stated in the protocol are what we typically see during a conjugation reaction and should be used as guidelines.

13. Does the sodium azide in the initial antibody solution interfere with the conjugation reaction?

    No. Sodium azide will be removed from the reduced antibody on the NAP-5 column and thus should not interfere with the conjugation reaction.

14. My protein is not an antibody and does not have free sulfhydryls or disulfides. Can I use your kit to conjugate Qdot® nanocrystals to my protein?

    An alternative chemistry to antibody reduction using DTT must be used to introduce sulfhydryls. We have tried SATA activation with subsequent deprotection; optimized reaction conditions will have to be worked out by the end-user.

15. What is the preferred buffer for antibody reduction?

    We recommend the use of phosphate buffered saline, pH 7.4 (PBS). Other buffers of similar pH values should work equally well.

16. My antibody concentration is less than 1 mg/ml or greater than 1 mg/ml. Must I concentrate/dilute before performing the conjugation?

    We typically conjugate with a starting antibody concentration of 1 mg/ml, but this may not be a necessity. We have found that using the recommended concentration and volume of antibody produces conjugates with high efficiency and minimal aggregation. The total volume of the antibody reduction reaction should be less than 500 µl, however, as this is the limit for use of the supplied NAP-5 columns.

17. Is it necessary for my antibody to be purified?

    Yes. Ascites, BSA, and/or other proteins will interfere with the conjugation and should therefore be removed.

18. I forgot to quench the reaction mixture by addition of β-mercaptoethanol. How will that affect the product?

    β-mercaptoethanol reacts with any remaining maleimide functional groups on the surface of Qdot® nanocrystals and converts them to non-reactive derivatives. This step significantly reduces the possibility of aggregation of the conjugate in the subsequent processing steps. Omission of this step may cause an increase in the aggregation, which in turn can adversely affect the performance of the conjugate.

19. Do I have to remove any unconjugated antibody after the conjugation reaction?

    Any unconjugated antibody remaining in the reaction mixture will compete with the Qdot® nanocrystal conjugate for binding to their specific antigen. The fluorescent signals will be reduced. The degree of this reduction may be assay-specific. Therefore, for best results, we strongly recommend a final purification step for unconjugated antibody removal.

20. Is there any way to determine the amount of unconjugated antibody remaining in the final product?

    The best way to do this is by analyzing the sample by size-exclusion HPLC. If the protocol provided with the kit is followed, only trace amounts of unconjugated antibody will be contained in the first fraction off the size exclusion column.

21. Why isn't all of the final conjugate collected in one fraction off the final purification column?

    For a whole antibody, the size exclusion column used does not provide baseline separation between conjugate and unconjugated antibody, thus some unconjugated antibody may elute with the conjugate. Fractionating reduces the chances of unconjugated antibody contaminating the final conjugate. Antibody fragments {F(ab')2} are cleanly removed from the final conjugate and fractionation is not necessary.

22. Can I use ultrafiltration instead of your column to purify my final conjugate?

    We have found that using ultrafiltration requires multiple rounds of purification and increases aggregation in the final conjugate.

23. Can I add a preservative to the final conjugate solution?

    We have not extensively explored the use of many preservatives, but sodium azide does not affect conjugate performance.

Qdot® Nanocrystal Conjugate Basics

Structure

Qdot® nanocrystals consist of nanometer-scale crystals of semiconductor material (CdSe), which have been 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 655 nm. This core-shell material (see 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 an antibody (See Figure 1B).

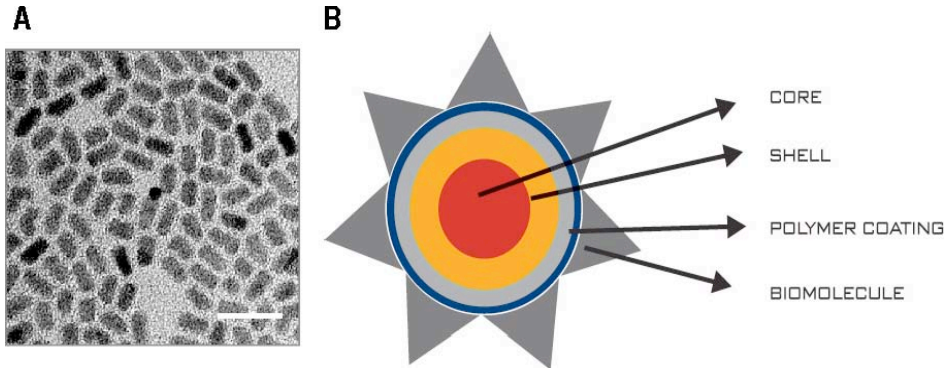

Figure 1: A: Transmission electron microscope image of core-shell Qdot® nanocrystal at 200,000x magnification. Scale bar = 20 nm.
B: Schematic of the overall structure of a Qdot® nanocrystal conjugate. The layers represent the distinct structural elements of the Qdot® nanocrystal conjugate, and are roughly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

Optical Properties

The optical properties of these conjugates are different than those of typical dye molecule-biomolecule conjugates. The color of light that the Qdot® nanocrystal component of a conjugate emits is strongly dependent on the size of the core, creating a common platform of labels from the green to the red, all manufactured from the same underlying semiconductor material.[1] The size of the Qdot® nanocrystals is tightly controlled in the production process, resulting in materials with narrow and symmetric emission spectra, that are extremely bright and photostable. These properties are exploited in a variety of immunofluorescence techniques, and provide substantially stronger and more stable signals than are attainable with conventional immunofluorescent labels.[2] 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.

Unique Detection Requirements of Qdot® Nanocrystal-Antibody Conjugates

General Spectral Properties

Typical fluorescence 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 fluorescence dyes reflect this characteristic.[4] Qdot® nanocrystals have absorbance spectra that increase dramatically at wavelengths shorter than the emission wavelength (Figure 2). These unique spectral properties are due to the semiconductor that makes up the core of a conjugate made with a Qdot® nanocrystal.[1] In spite of the broad absorbance, the emission is narrow, symmetric, and independent of the excitation wavelength; so whether exciting at 595 nm or at 400 nm, the shape of the emission remains the same, while the intensity is approximately 5-fold higher with 400 nm excitation. This absorbance and hence excitation at shorter wavelengths, together with fixed emission results in a large "apparent Stokes shift" that improves sensitivity by reducing auto-fluorescence, and greatly simplifies the multiplexed detection using conjugates made of multicolor Qdot® nanocrystals. See Appendix 3 for extinction coefficients of the different materials.

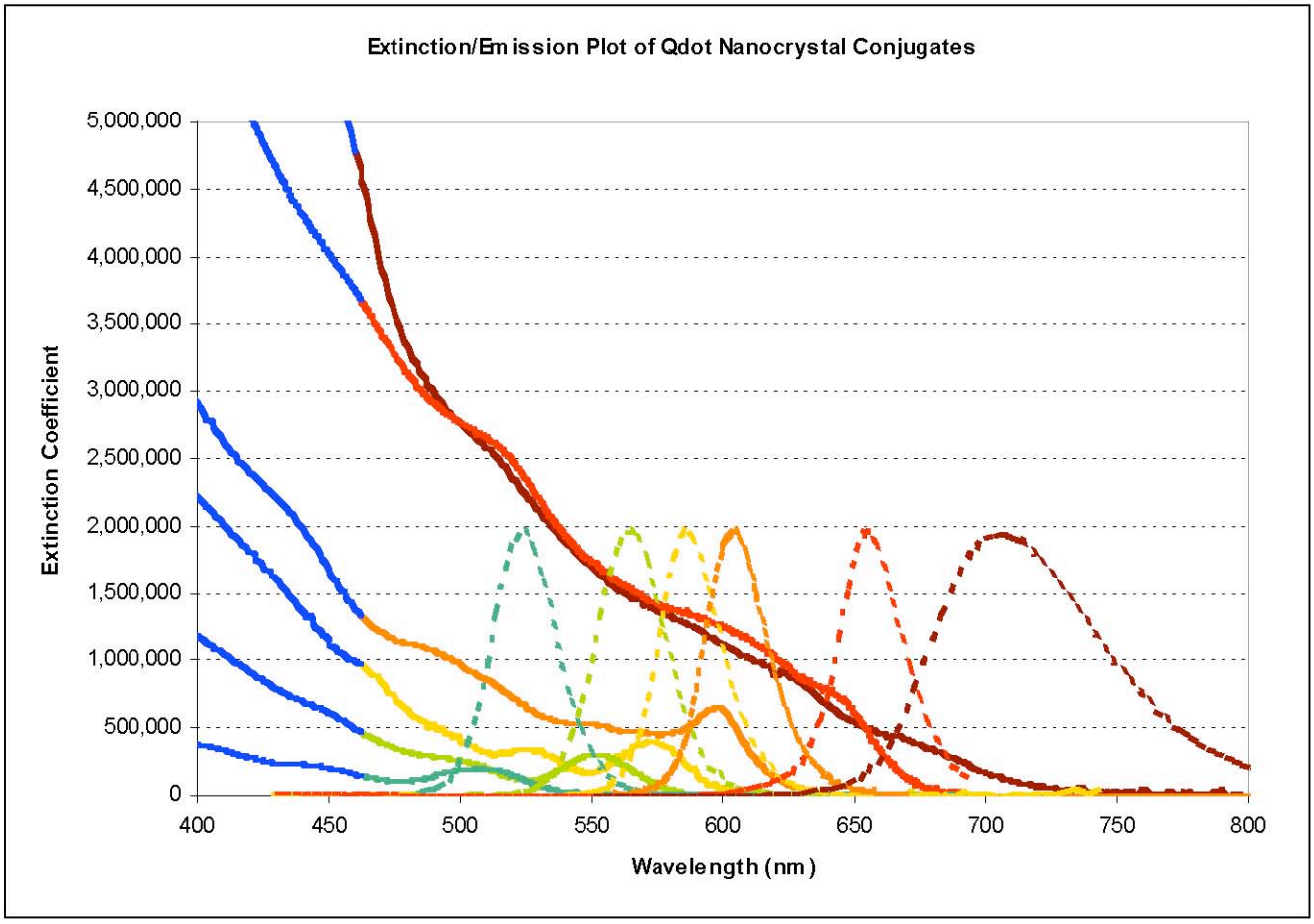

Figure 2: Typical absorbance and emission spectra of Qdot® 705 conjugate (brown curves), Qdot® 655 conjugate (dark red curves), Qdot® 605 conjugate (orange curves), Qdot® 585 conjugate (yellow curves), Qdot® 565 conjugate (chartreuse curves), Qdot® 525 conjugate (green curves). The inlaid blue lines on the absorbance represent a broad window of absorbance that will excite the materials more efficiently than a single wavelength excitation. Such excitation can be achieved through the use of a short-pass excitation filter. (See text below)

Optical Filter Selection

The broad absorbance spectrum of the Qdot® nanocrystal is ideally suited to allow unique possibilities in lamp-based fluorescent imaging systems and plate readers that have broad excitation filters in the UV-blue region of the spectrum. The integrated absorbance across an excitation band can be substantially higher than any single wavelength value, i.e. laser excitation, (Figure 2—illustrated by the blue curves). Using a lamp with a short-pass filter allows highly efficient excitation of the Qdot® nanocrystals, and can ultimately be combined with all colors of conjugates made with Qdot® nanocrystals for efficient and simple multiplexing analysis. In order to achieve the optimal signal from a conjugate made with a Qdot® nanocrystal, we recommend using a custom filter set. Custom filter sets are available from Omega Optical (www.omegafilters.com).

Qdot® nanocrystals can also be viewed through some standard filter sets, albeit with lower detection efficiency and reduced brightness. For example, three Omega Optical standard filter sets capable of detecting Qdot® 605 nanocrystals are XF140-2 (Alexa Fluor® 633 & Alexa Fluor® 647), XF70 (Alexa Fluor® 660 & Cy®5), and XF141-2 (Cy5.5). Visualization of conjugates made with Qdot® nanocrystals 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® nanocrystals. Using a custom filter set, Qdot® 605 nanocrystal 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 (See Figure 3). Qdot® nanocrystal-optimal filters and standard filter sets are available from many different filter manufacturers. Appendix 1 below illustrates some common filter sets and the optimal filter set recommendations for the available Qdot® nanocrystals. Use of the optimal filter set is tied quite tightly to attaining optimal signal and sensitivity in your experiments. Please consult our website (www.invitrogen.com) for detailed technical notes and examples of how to set up specific instruments to detect optimally conjugates made with Qdot® nanocrystals.

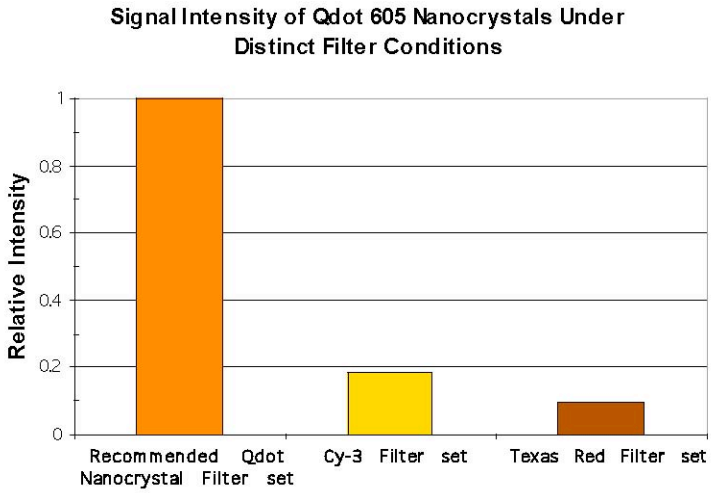

Figure 3: Detection of Qdot® nanocrystals on tissue sections with recommended and standard filter sets. Mouse kidney sections were stained with Qdot® 605 Streptavidin conjugate, and then images were collected on a Nikon epi-fluorescence microscope in 16 bit capture mode. The mean fluorescence of positively stained samples was extracted using Scion Image software. The recommended Qdot® nanocrystal filter set included a 460 nm short pass exciter, a 475 nm dichroic, and a 605/20 nm band pass emitter. The Cy-3 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

Appendix 1: Filter Recommendations for Qdot® Nanocrystals

	Omega Optical (www.omegafilters.com)	
Color	**Optimal filter sets**	**Usable filter sets**
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)
705**	**XF306 Qdot705 filter set** (Exciter 1: 425DF45 or Exciter 2: 415WB100/ Dichroic: 475DCLP/ Emitter: 710AF40)	XF140-2, XF70, XF110-2, XF141-2, XF48-2
655	**XF305 Qdot655 filter set** (Exciter 1: 425DF45 or Exciter 2: 415WB100/ Dichroic: 475DCLP/ Emitter: 655WB20)	XF102-2, XF40-2, XF42, XF45
605	**XF304 Qdot605 filter set** (Exciter 1: 425DF45 or Exciter 2: 415WB100/ Dichroic: 475DCLP/ Emitter: 605WB20)	XF108-2, XF102-2, XF103-2
585	**XF303 Qdot585 filter set** (Exciter: 1 425DF45 or Exciter 2: 415WB100/ Dichroic: 475DCLP/ Emitter: 585WB20)	XF101-2, XF137-2, XF152-2
565	**XF302 Qdot565 filter set** (Exciter 1: 425DF45 or Exciter 2: 415WB100/ Dichroic: 475DCLP/ Emitter 565WB20)	XF104-2, XF105-2
525	**XF301 Qdot525 filter set** (Exciter 1: 425DF45 or Exciter 2: 415WB100/ Dichroic: 475DCLP Emitter: 525WB20)	XF100-3, XF100-2, XF115-2, XF89-2
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

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

Appendix 2: Additional Usable Filter Sets for Qdot® Nanocrystals

	Chroma Technology (www.chroma.com)	
Color	**Usable filter sets**	**Optimal filter sets**
800	Cy7 (41009), Li-Cor for IRDye 800 (41037), Cy7 (SP106)	**Optimized filter sets will be available soon**
705	Cy5 Longpass (41024), Cy5 (41008), Cy5 narrow excitation (41033), Cy5.5 (41023), Alexafluor 680 (41042), Cy5.5 (redshifted; 41022)	**Optimized filter sets will be available soon**
655	Texas Red (41004), Propidium Iodide (41005), Fura Red (31012), Chlorophyll (31017), Allophycocyanin (31006)	**Qdot 655 filter set** (20 nm EM; 32011) (460SPUV/475DCXRU/D655/ 20nm) **Qdot 655 filter set** (40 nm EM; 32012) (460SPUV/475DCXRU/D655/ 40nm)
605	Cy-3 narrow excitation (41007a), Texas-red/Cy 3.5 (31004), TRITC (41002, 41002a, 41002b), Ethidium Bromide (41006)	**Qdot 605 filter set** (20 nm EM; 32003) (460SPUV/475DCXRU/D605/ 20nm) **Qdot 605 filter set** (40 nm EM; 32007) (460SPUV/475DCXRU/D605/ 40nm)
585	R-PE (41003), Rhodamine LP (41032, FITC/PI (41016)	**Qdot 585 filter set** (20 nm EM; 32004) (460SPUV/475DCXRU/D585/ 20nm) **Qdot 585 filter set** (40 nm EM; 32008) (460SPUV/475DCXRU/D585/ 40nm)
565	Eosin (41011), Cascade Yellow (31038), JP2(YGFP with EGFP31040, Auramine (31015)	**Qdot 565 filter set** (20 nm EM; 32005) (460SPUV/475DCXRU/D565/ 20nm) **Qdot 565 filter set** (40 nm EM; 32009) (460SPUV/475DCXRU/D565/ 40nm)
525	FITC/RSGFP/BODIPY/Fluo3/DiO (41001), FITC/RSGFP Longpass (40012), BFP to GFP FRET (31032), BFP to GFP FRET wide excitation (31034), GFP wide blue excitation (31054)	**Qdot 525 filter set** (20 nm EM; 32006) (460SPUV/475DCXRU/D525/ 20nm) **Qdot 525 filter set** (40 nm EM; 32010) (460SPUV/475DCXRU/D525/ 40nm)
All colors	UV (11000V2), Blue/Violet (11003V2), UV/Violet (11011V2) *	**Qdot Multiple Emission Set** (71014) (460SPUV, 475DCXRU, D525/20nm, D605/20nm, D565/20nm, D585/20nm)
* For viewing multiple colors of Qdot® nanocrystals through microscope eye pieces.		

Appendix 3: Extinction Coefficient of Qdot® Nanocrystals 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 $M^{-1}cm^{-1}$	N/A
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® 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	12,900,000 $M^{-1}cm^{-1}$	8,300,000 $M^{-1}cm^{-1}$	3,000,000 $M^{-1}cm^{-1}$	2,000,000 $M^{-1}cm^{-1}$

Appendix 4: References

1. 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 h through k describe Qdot® nanocrystals and FRET.
   a. Alivisatos, AP. Less is More in Medicine. Scientific American. 2001. 285(3):66-73.
   b. Alivisatos, AP. Perspectives on the Physical Chemistry of Semiconductor Nanocrystals. Journal of Physical Chemistry B. 1996. 100(31): 13226-13239.
   c. Murray, CB, Norris, DJ, and Bawendi, MG. Synthesis and Characterization of Nearly Monodisperse CdE Semiconductor Nanocrystallites. Journal of the American Chemical Society. 1993. 115(19): 8706-8715.
   d. Norris, DJ, Bawendi, MG. Measurement and assignment of the size-dependent optical spectrum in CdSe Qdot® nanocrystals. Physical Review B. 1996. 53(24):16338-16346.
   e. Hines, MA, Guyot-Sionnest, P. Synthesis and Characterization of Strongly Luminescing ZnS capped CdSe Nanocrystals. Journal of Physical Chemistry. 1996. 100(2):468-471.
   f. Dabbousi, BO, et al. (CdSe)ZnS Core-Shell Qdot® nanocrystals: Synthesis and Characterization of a Size Series of Highly Luminescent Nanocrystallites. Journal of Physical Chemistry B. 1997. 101(46): 9463-9475.
   g. Peng, X, Schlamp, MC, Kadavanich, AV, Alivisatos, AP. Epitaxial Growth of Highly Luminescent CdSe/CdS Core/Shell Nanocrystals with Photostability and Electronic Accessibility. Journal of the American Chemical Society. 1997. 119(30): 7019-7029.
   h. Willard, D. M.; Carillo, L. L.; Jung, J.; Van Orden, A. "CdSe-ZnS Qdot® nanocrystals as Resonance Energy Transfer Donors in a Model Protein-Protein Binding Assay," Nano Lett. 2001, 1, 469-474.
   i. Clapp, A. R.; Medintz, I. L.; Mauro, J. M.; Fisher, B.; Bawendi, M. G.; Mattoussi, H. "Fluorescence Resonance Energy Transfer Between Qdot® nanocrystal Donors and Dye-Labeled Protein Acceptors," J. Am. Chem. Soc. 2004, 126, 301-310.
   j. Medintz, I. L.; Clapp, A. R.; Mattoussi, H.; Goldman, E. R.; Fisher, B.; Mauro, J. M. "Self-Assembled Nanoscale Biosensors Based on Qdot® nanocrystal FRET Donors," Nature Mater. 2003, 2, 630-638.
   k. Jares-Erijman, E. A.; Jovin, T. M. "FRET Imaging," Nature Biotech. 2003, 21, 1387-1395.

2. A number of references have appeared recently that describe the biological properties of some Qdot® nanocrystals 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.
   a. Bruchez, MP, et al. Semiconductor Nanocrystals as Fluorescent Biological Labels. Science. 1998. 281(5385): 2013-2016.
   b. Chan, WC and Nie, S. Qdot® nanocrystal Bioconjugates for Ultrasensitive Nonisotopic Detection. Science. 1998. 281(5385): 2016-2018.
   c. Rosenthal, SJ, et.al. Targeting Cell Surface Receptors with Ligand Conjugated Nanocrystals. Journal of the American Chemical Society. 2002. 124(17):4586-4594.
   d. Akerman, ME, et.al. Nanocrystal Targeting In-Vivo. Proceedings of the National Academy of Sciences. 2002. 99(20):12617-12621.
   e. Dubertret, B. et.al. In vivo imaging of Qdot® nanocrystals encapsulated in phospholipid micelles. Science. 2002. 298(5599): 1759-1762.
   f. Wu, X. et al. Immunofluorescence Labeling of Cancer Marker her-2 and Other Cellular Markers with Semiconductor Qdot® nanocrystals. Nature Biotechnology. 2003. 21(1):41-46.
   g. Jaiswal, J et.al. Long-term multiple color imaging of live cells using Qdot® nanocrystals. Nature Biotechnology. 2003. 21(1):47-51.

3. Hermanson, GT. Bioconjugate Techniques, Academic Press, 1996.
4. Lakowicz, J. Principles of Fluorescence Spectroscopy. Kluwer Academic Publishing, 1999.
5. Colligan, et. al. Current Protocols in Immunology. J. Wiley, Annually Updated, 2002.