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# Qdot® Biotin Conjugates User Manual

Quantum Dot
invitrogen nanocrystal technologies

Molecular Probes™
invitrogen detection technologies

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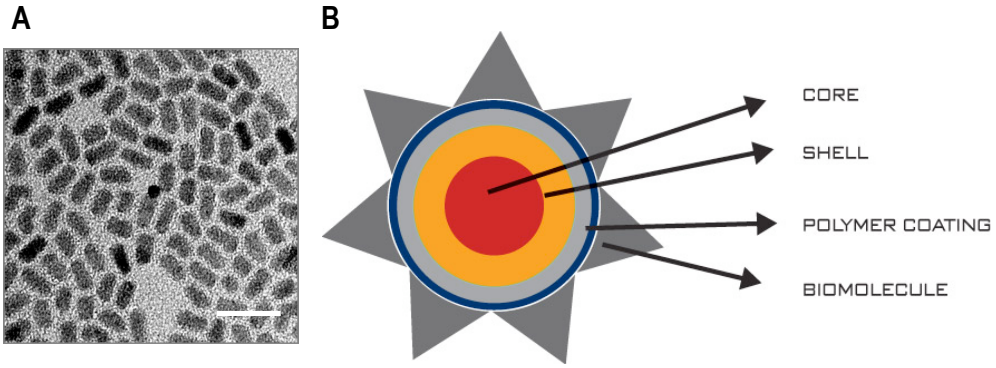



**Figure 1**: **A**: Transmission electron microscope image of core-shell Qdot® nanoparticles at 200,000x magnification. Scale bar = 20 nm. **B**: Schematic of the overall structure of a Qdot® Biotin Conjugate. The layers represent the distinct structural elements of the Qdot nanocrystal conjugates,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

## Qdot® Conjugate Basics

### Structure

The Qdot® Biotin Conjugate is made from a nanometer-scale crystal of semiconductor material (CdSe), which has 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 605 nm (Cat. # Q10301MP) or 655 nm (Cat. # Q10321MP). This core-shell material (see Figure 1A above) 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 has been directly coupled to Biotin (See Figure 1B above). The Qdot Biotin Conjugate is the size of a large macromolecule or protein (~10-12 nm). This conjugate is supplied with an incubation/dilution buffer optimized for staining in a variety of immunofluorescence applications.

### Optical Properties

The optical properties of these 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[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 can result in substantially better results 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.

### 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 the Qdot® Incubation Buffer, or with some other blocking buffers (results may vary in alternate buffer systems). Qdot nanocrystals have been coupled to Biotin directly through a carbodiimide-mediated coupling reaction[3]. This yields a material with a high loading of Biotin on the surface (typically 5-7 Biotin molecules/Qdot conjugate), which results in Qdot Biotin Conjugates with high specific biological activity.

The probes should, however, be used as if there were one Biotin per quantum dot, which is the concentration as indicated on the tube. Though one quantum dot is capable of bridging multiple antigens, the dominant binding mode is one Qdot conjugate per analyte if the assay is carried out at a saturating concentration. (See Figure 2.)

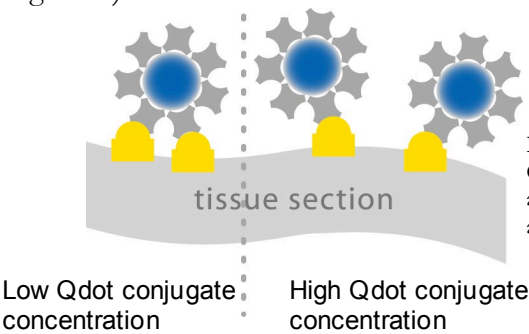



**Figure 2:** Impact of working at the appropriate concentration range for Qdot Biotin Conjugates. Due to the multivalency of the conjugates, use of conditions below the appropriate saturation concentration may result in artificially reduced signals due to antigen bridging with a single quantum dot.

## Unique Detection Requirements of Qdot Biotin 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]. Quantum dots 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[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. 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 further Qdot Biotin Conjugates. See Appendix 2 for the extinction coefficients of the conjugate.

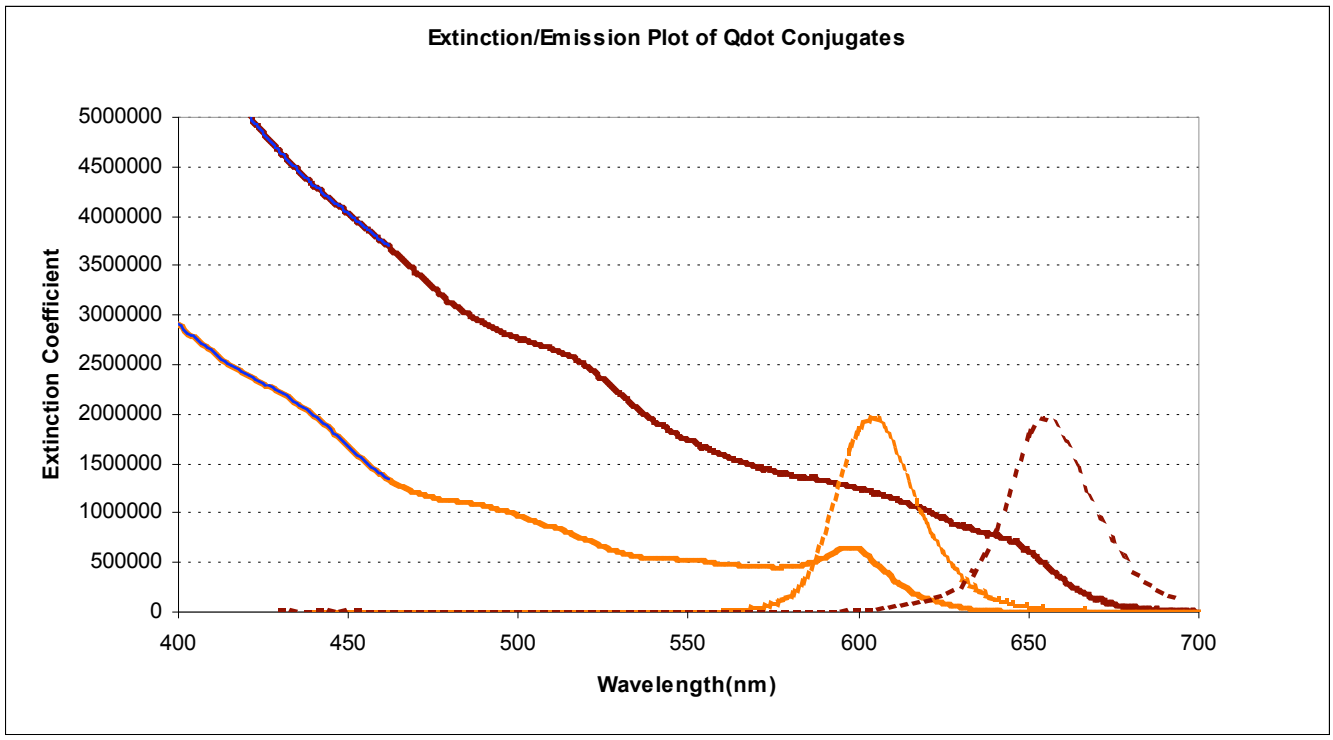



**Figure 3:** Typical absorbance and emission spectra of the Qdot 605 Biotin Conjugate (orange curves) and Qdot 655 Streptavidin Conjugate (dark red curves). The inlaid blue line on the absorbance represent a broad window of absorbance that will excite the material more efficiently than a single wavelength 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 quantum dot allows a unique possibility in lamp-based fluorescent microscopes. The integrated absorbance across a broader excitation band is substantially higher than any single wavelength value (Figure 3—illustrated by the blue curve). Using a lamp with a short-pass filter allows highly efficient excitation of the quantum dots, and can ultimately be combined with all colors of Qdot conjugates for efficient and simple multiplexing analysis. In order to achieve the optimal signal from the Qdot Biotin Conjugates, we recommend using a custom filter set. All comparative data was gathered using Chroma filters, which can be purchased separately through a special order from Chroma Technology Corporation (www.chroma.com). Custom filter sets are also available from Omega Optical (www.omegafilters.com).

The Qdot® Biotin Conjugates can also be viewed through some standard filter sets, albeit with lower detection efficiency and reduced brightness. For example, two Chroma standard filter sets capable of detecting Qdot 605 conjugates are 41007a (Cy®3-narrow excitation) and 31004 (Texas Red®/Cy®3.5). 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 Biotin 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®/Cy®3.5 filter set. Table 1 below illustrates some common filter sets and the optimal filter set recommendations for the available Qdot Biotin Conjugates. Use of the optimal filter set is tied quite tightly to attaining optimal signal and sensitivity in your experiments. Please contact Customer Service for more details (800-438-2209).

**Table 1:** Filter recommendations for Qdot Biotin Conjugates

| | Chroma Technology (www.chroma.com) | | Omega Optical (www.omegafilters.com) | |
|---|---|---|---|---|
| **Color** | **Usable filter sets** | **Optimal filter sets** | **Usable filter sets** | **Optimal filter sets** |
| **655** | Texas Red (41004), Propidium Iodide (41005), Fura Red (31012), Chlorphyll (31017), Allophycocyanin (31006) | **Qdot 655 filter set** (20 nm EM; 32011) (460SPUV/475DCXRU/D655/20 nm) **Qdot 655 filter set** (40 nm EM; 32012) (460SPUV/475DCXRU/D655/40 nm) | XF102-2, XF40-2, XF42, XF45 | **XF305 Qdot655 filter set** (Exciter 1 435DF70 or Exciter 2 415WB100/ Dichroic 470AGLP/ Emitter 655DF20) |
| **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) | XF108-2, XF102-2, XF103-2 | **XF304 Qdot605 filter set** (Exciter 1 435DF70 or Exciter 2 415WB100/ Dichroic 470AGLP/ Emitter 605DF20) |
| **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) | XF129-2, XF130-2 | **XF300 Qdot filter set** |

***For looking down the microscope to simultaneously visualize multiple colors**

## PLEASE READ ENTIRE PROTOCOL BEFORE STARTING

Invitrogen has conjugated Biotin to the Qdot nanocrystals to allow use of the materials in a wide variety of labeling applications. New protocols and application notes will be posted on our website as they become available (www.invitrogen.com). For a flowchart that details the steps in this staining protocol, please consult Appendix 4.

### Materials

- Qdot® Biotin Conjugate
- Qdot® Streptavidin Conjugate
- 96-well low binding plate
- Streptavidin *
- Tris-buffered saline (TBS)
- Blocking buffer (TBS containing 0.05% Tween-20)
- *Optional:* Biotin **

*** Streptavidin was used at 0.1mg/ml in 100 mM Carbonate, pH 10. User can test optimal concentration range or use pre-coated Streptavidin or Avidin plate.**

**** Biotin in the concentration range of 1 mM – 10 mM was used to suppress the specific signal.**

**Notes:**
1. Centrifuge at 5,000-10,000 x g, for 5-10 min. reserving the supernate, prior to using the material.
2. Some PAP pens can quench the signal from the quantum dots. If your protocol requires the use of a PAP pen, we recommend the ImmEdge Hydrophobic Barrier Pen (H-4000) from Vector Labs.

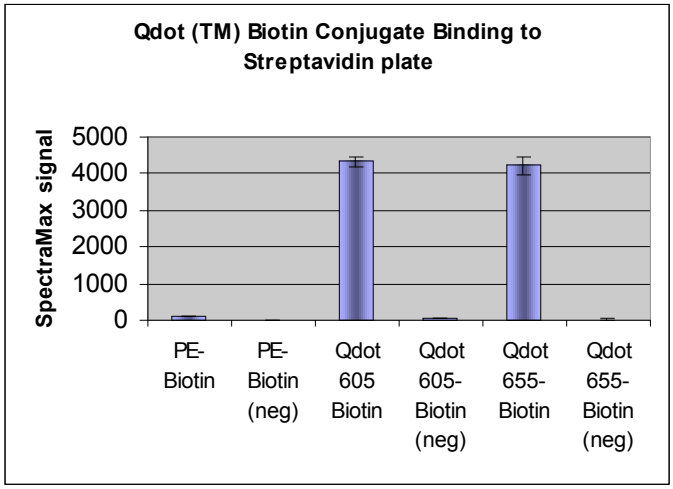
## Procedure

All steps of the procedure should be performed at room temperature. Parafilm or protective lids may be used to cover the plate during incubation times.

1) Dispense 50 µl of Streptavidin per sample well of uncoated plate.
2) Incubate at room temperature overnight.
3) Discard Streptavidin and dispense 50 µl of blocking buffer per sample well.
4) Incubate for 20 min.
5) Rinse 2X with TBS.
6) *Optional:* Block with 50 µl Biotin at the appropriate concentration for any negative control for 30min. Use TBS for positive sample wells.
7) Dilute the Qdot® Biotin Conjugate to the appropriate concentration (saturated at 6 nM, as tested) and dispense per sample well, maintaining a volume close to 50 µl.
8) Mix well and incubate for 1 hour.
9) Rinse 2X with TBS.
10) Dispense 50 µl of TBS per sample well and collect data on florescent plate reader and the appropriate excitation wavelength (250 nm ex, 605 or 655 as detected).

For *Signal Amplification*, repeat Steps 7 – 10 with Qdot® Streptavidin Conjugate. Alternate between Qdot® Biotin Conjugate and Qdot® Streptavidin Conjugate for more signal amplification.

## Representative Data



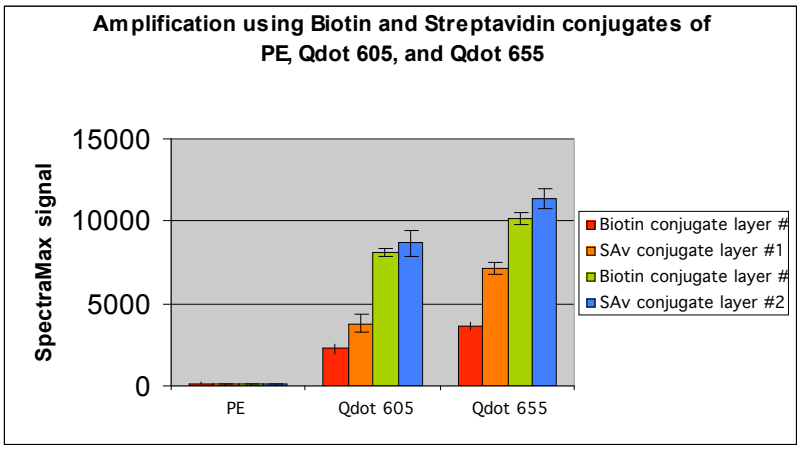




**Figure 4**: **Fluorescence intensity comparison with and without amplification.** Streptavidin was bound to a polystyrene fluorescence plate, and then detected with Qdot 605 Biotin, Qdot 655 Biotin, and PE-Biotin (upper panel). The signal was detected under optimal conditions for each probe, and the signal from the Qdot Biotin Conjugate is ~50 fold brighter than Phycoerythrin-Biotin. The bright signals can be developed further with sequential amplification using alternating incubation with Qdot-Biotin and Qdot-Streptavidin Conjugates (lower panel).

Biotinylated conjugates (Phycoerythrin from Molecular Probes, Inc.) were incubated with the plate at 6 nM concentration, then washed. Negative controls were blocked with 10 mM biotin to prevent specific binding. (Excitation 250 nm Qdot Biotin Conjugates; excitation 544 nm PE-Biotin)

## Notes

1. The optimal signal-to-background ratio is obtained from an assay that has been optimized with respect to all of the probe concentrations. The optimal dilution of Qdot Biotin Conjugate should be experimentally determined for each application to obtain the highest signal-to-background ratio.

## Appendix 1: **Troubleshooting and Frequently Asked Questions**

The properties of Qdot 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. Which buffers are compatible with the Qdot Biotin Conjugates?

Although we have not tested them directly, we expect the Qdot Biotin Conjugates to exhibit the same buffer compatibility and stability as the Qdot Streptavidin Conjugates. 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, 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 precipitation, 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 in 0.05% concentrations. In contrast, gelatin and dextran sulfate were both found to promote aggregation of the Qdot Streptavidin Conjugates at 0.05% concentrations, and should be avoided in labeling applications. In general, we recommend storage of the Qdot conjugates at the concentration at which it is shipped, rather than at a high dilution. Storage of materials at working dilution over longer periods of time may result in substantial performance degradation.

2. Which buffers can I use in place of the Qdot Incubation Buffer provided?
    - Qdot conjugates have higher nonspecific binding in buffers that are not optimized for use with the materials. We have had successful staining results in a variety of buffer conditions, including TBS, PBS, RPMI media, and others, but have found that the performance in the Incubation Buffer is generally predictable and stable. We are developing a number of application notes with details for a variety of applications, which can be found on the website (www.qdots.com), as they become available.

3. Are the quantum dots toxic?
    - We have not investigated the toxicity of the Qdot Biotin 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 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.

4. How should I dispose of the Qdot conjugate?
    - The Qdot 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.

5. Do the quantum dots undergo FRET, or quench when they are in close proximity?
    - We have not systematically investigated the energy transfer properties of the quantum dots, though the quantum dots may have useful properties as both energy transfer donors and acceptors. We have investigated the fluorescence of Qdot 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.

## No signal:

1. Confirm imaging/detection setup suitability.

    - Make sure that you are using an appropriate filter set to detect the labeling signals. Please consult Table 1 for a list of appropriate and optimal filters for the Qdot Conjugates. Contact Customer Service (800-438-2209) for more detail on particular filter set requirements for distinct systems.

2. Check to see that Qdot conjugate is luminescent.

    - Qdot conjugates will normally fluoresce brightly under a hand-held ultraviolet lamp (long wave, such as the type used to visualize ethidium bromide on agarose gels). 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 Biotin Conjugates do not appear to fluoresce under the long wave UV excitation, please contact Technical Support for a replacement. (probestech@invitrogen.com or 800-438-2209)

3. Confirm the specificity and titer of primary antibody.

    - Make sure the antibody will recognize the intended targets. Make sure 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[5].

4. PAP pen ink may bleach staining signals.

    - Use an alternate method for isolating target areas on the slide.

## High Background:

1. Use the Qdot Incubation Buffer.

    - The included buffer is formulated specifically to achieve improved signal to noise ratios in most immunohistochemical applications using the Qdot Biotin Conjugate. Alternate buffers can result in more variable staining results, and in particular may increase the background staining. However, some **specific applications** may need other buffer conditions. For example, we have observed that using a 5X buffer dilution of the 10X Sigma Blocking Buffer (Cat # B6429) works better in staining acetone-fixed human epithelial cells. Please see the

protocol "Double-labeling Using Qdot® Streptavidin Conjugates" (available at www.invitrogen.com).

2. Grainy staining or clumps of fluorescent material appears in the background.

    - Occasionally the BSA within the incubation buffer shows slight aggregation over time. It is necessary to remove this aggregate prior to labeling the sample with the Qdot conjugate. Spin down the incubation mixture before addition to the sample. This can be accomplished by spinning the samples on a benchtop centrifuge (Eppendorf 5415) at 5000 xg for 2 minutes. The materials can also be passed over a 0.2 μm spin filter unit prior to addition to the sample for staining to remove microscopic precipitates. If you are using a buffer that is different than the Qdot Incubation Buffer, this behavior can often be attributed to higher levels of NaCl in the incubation buffer, and may not be easily fixed with filtration. Reduce the overall salt concentration to reduce this problem.

3. Optimize concentration of Qdot conjugate.

    - Just as titration of primary and secondary antibodies are necessary to achieve optimal specific signal in immunohistochemical applications, adjustment of the level of the final probe should be optimized for each conjugate. In general, concentrations at or slightly below saturation should have the optimal signal/background ratio, while concentrations substantially higher than saturation will compromise the assay with higher background levels.

## Appendix 2: Extinction Coefficient of Qdot Biotin Conjugates at Common Excitation Wavelengths

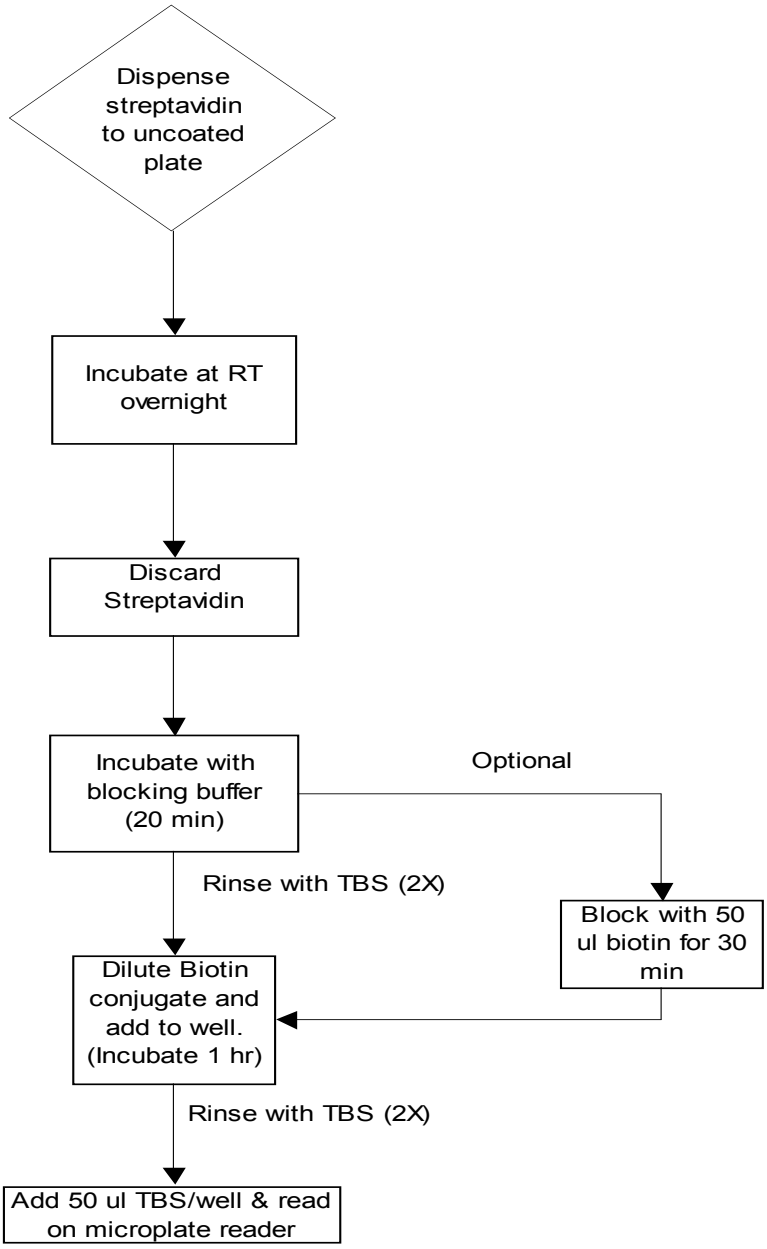
| Product | 350 nm | 405 nm | 488 nm | 532 nm |
|---|---|---|---|---|
| Qdot 605 Biotin Conjugate | 4,400,000 $M^{-1}cm^{-1}$ | 2,800,000 $M^{-1}cm^{-1}$ | 1,100,000 $M^{-1}cm^{-1}$ | 580,000 $M^{-1}cm^{-1}$ |
| Qdot 655 Biotin Conjugate | 9,100,000 $M^{-1}cm^{-1}$ | 5,700,000 $M^{-1}cm^{-1}$ | 2,900,000 $M^{-1}cm^{-1}$ | 2,100,000 $M^{-1}cm^{-1}$ |

## Appendix 3: 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.
    a. Alivisatos, AP. Less is More in Medicine. *Scientific American*. 2001. 285(3):66-73.
    b. Alivisatos, A.P. 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 quantum dots. *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 Quantum Dots: 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.
2. A number of references have appeared recently that 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.
   a. Bruchez, MP, *et al.* Semiconductor Nanocrystals as Fluorescent Biological Labels. *Science*. 1998. 281(5385): 2013-2016.
   b. Chan, WC and Nie, S. Quantum Dot 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 quantum dots 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 Quantum Dots. *Nature Biotechnology*. 2003. 21(1):41-46.
   g. Jaiswal, J *et.al.* Long-term multiple color imaging of live cells using quantum dots. *Nature Biotechnology*. 2003. 21(1):47-51.
3. Hermanson, Bioconjugate Techniques, *Academic Press*, 1996.
4. Lakowicz, J. Principles of Fluorescence Spectroscopy. *Kluwer Academic Publishing*, 1999.
5. Colligan, *et.a*l. Current Protocols in Immunology. *J. Wiley*, Annually Updated, 2002.
6. E. Harlow and D. Lane, in "Using Antibodies, A Laboratory Manual", Cold Spring Harbor Laboratory Press (1999).
7. S. Sheikh and A. Mulchandani, "Continuous-flow Fluoro-immunosensor for Pactitaxel Measurement", Biosensors and Bioelectronics, **16**, 647-652 (2001).
8. T. Tanaka and T. Matsunaga, "Fully Automated Chemiluminescence Immunoassay of Insulin Using Antibody-Biotin-Bacterial Magnetic Particle Complex", Anal. Chem., **72**, 3518-3522 (2000).
9. P. Peluso et al., "Optimizing Antibody Immobilization Strategies for the construction of Protein Microarrays", Anal. Biochemistry, **312**, 113-124 (2003).

**Appendix 4: Plate Assay Flowchart**



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