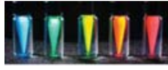
Use of Qdot® Nanocrystal Conjugates with Standard Sample Preparation Reagents



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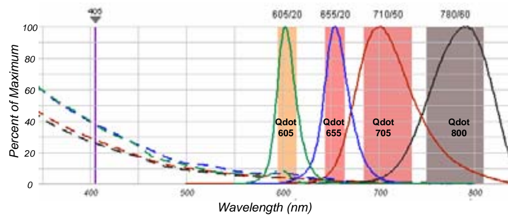
Introduction

Researchers are using Qdot nanocrystal conjugates of anti-leukocyte antibodies in their multi-color panels, and there have been occasional reports of loss of Qdot nanocrystal fluorescence across a variety of staining protocols. We have surveyed a number of standard blood cell procedures protocols for compatibility with Qdot nanocrystal conjugate staining. For comparison, stains in this study were done on human leukocytes stained with Qdot 655-anti-human CD4 and run on a BD LSR II cytometer. We demonstrate compatibility of Qdot nanocrystal antibody conjugates with a number of available leukocyte sample preparation reagents: Most erythrocyte lysis reagents, including Cal-Lyse™ reagent, Caltag™ High Yield Lyse, and ammonium chloride, can cause a decrease in nanocrystal fluorescence without loss of population resolution, as assessed by staining index. Specific lysis solutions, such as BD™ PhosFlow™ lyse, degraded Qdot fluorescence on stained cells. Aldehyde-containing fixatives, including FIX & PERM® solution A and 4% formaldehyde, caused a decrease in both positive and negative peak fluorescence, again without a decrease in staining index. When used with stained cells after formaldehyde fixation, permeabilizing reagents, including saponin, Triton® X-100, and methanol solutions, had little impact on Qdot nanocrystal fluorescence. Because of broad reagent compatibility, it should be possible to insert Qdot conjugates into standard staining protocols with no more than minor changes in sample preparation reagents.

Figure 1. Qdot Nanocrystal Properties

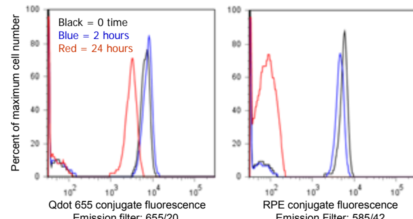
- Fluorescent nanometer-scale semiconductor crystallites: generally CdSe, encapsulated in ZnS
- Emission wavelength determined by the particle size; tunable from ~2-10 nm (±3%)
- Qdot nanocrystals are derivatized with amino-polyethylene glycol to control nonspecific binding and provide conjugation sites for antibody



Excitation (dotted) and emission (solid) characteristics of Qdot nanocrystal antibody conjugates demonstrate long effective Stokes shifts and symmetrical, relatively narrow emission peaks. Nanocrystals can be excited by every wavelength up to their emission. Therefore, a Qdot 655 nanocrystal excites best with UV or violet, but excites well enough with 488 and 633 nm lasers either to be used with those excitation lines or to require *cross-laser* compensation to subtract the nanocrystal signal from other fluors excited by these lasers.

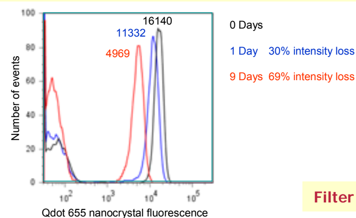
Left to right: Qdot 605, 655, 705, and 800 nanocrystals can be used in combination with conventional fluors, or can substitute for tandem dyes using Texas Red™, Cy®5, Cy5.5 and Cy7 dyes, respectively. Recommended band pass filters are shown for each nanocrystal.

Figure 2. Photostability of Qdot Nanocrystals



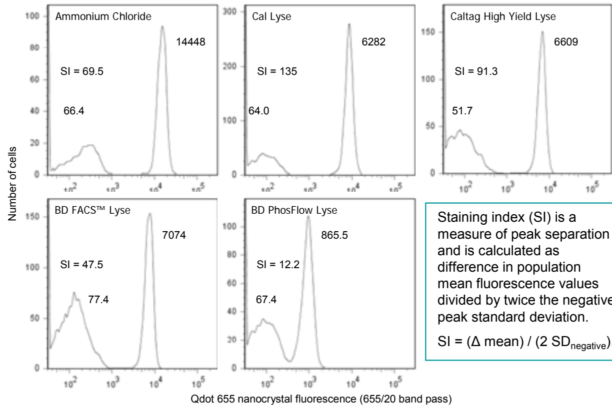
Anti-human CD4 conjugates were exposed to fluorescence light for the specified time before being used to stain human PBLs. Stained cells were held under fluorescent light for 0 time (black), 2 hours (blue), or 24 hours (red). Samples were analyzed using an LSR II cytometer with either 405 nm or 488 nm excitation and the specified emission filters. RPE fluorescence had completely bleached by 24 hours; nanocrystals retained fluorescence.

Figure 3. Stained Sample Stability



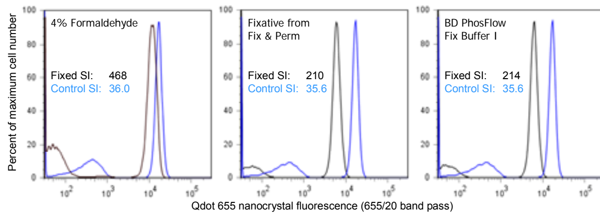
Human PBLs stained with Qdot 655-anti-human CD4 and fixed with formaldehyde, then held for the specified time period before analysis: black = 0 days, blue = 1 day, red = 9 days. Samples were analyzed using an LSR II cytometer with 405 nm excitation and a 655/20 emission filter. Histograms are smoothed and labeled with median fluorescence intensities.

Figure 4. Effect of Erythrocyte Lysis Reagents



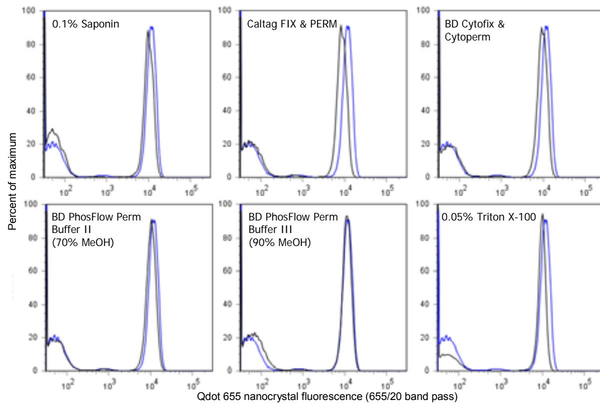
Human blood stained with Qdot 655-anti-human CD4, then lysed with the specified reagent. Samples were analyzed on an LSR II cytometer with 405 nm excitation and a 655/20 emission filter; gated on lymphocytes by scatter. Histograms are smoothed and labeled with geometric mean fluorescence values and staining index.

Figure 5. Effect of Aldehyde Fixatives



Human PBLs were stained with Qdot 655-anti-human CD4 before treatment with fixative. Samples were analyzed as in Figure 4. Histograms from fixed (black) and control (blue) samples are smoothed and labeled with staining indices.

Figure 6. Effect of Permeabilization Buffers after Fixation



Human PBLs stained with Qdot 655-anti-human CD4, fixed with formaldehyde and then permeabilized. Samples were run as in Figure 3. Histograms from fixed/permeabilized (black) and fixed only (blue) samples are smoothed.

Summary

- Qdot nanocrystal conjugates of monoclonal antibodies provide powerful and easy-to-use tools to extend the number of colors in multi-color flow cytometry panels
- Nanocrystal conjugates are compatible with standard sample preparation reagents and staining protocols
- Nanocrystal conjugates can be used efficiently on cytometers with either UV or violet excitation sources, with selection of appropriate filters
- As with other fluor conjugates in multicolor work, care must be taken in designing a reagent panel to minimize spectral overlap, with particular attention to the cross-laser excitation of nanocrystals
- Nanocrystals can also be used efficiently on cytometers with 488 nm or longer excitation sources as long as the nanocrystals are matched to available emission filters.

Filter Recommendations and Available Conjugates

Fluorophore	Emission maximum (nm)	Narrow band pass filter configuration		Broad band pass Filter configuration*	
Qdot 565 nanocrystal	565	555LP	565/20	557LP	560/40
Qdot 605 nanocrystal	605	570LP	605/20	595LP	605/40
Qdot 655 nanocrystal	655	640LP	655/20	640LP	660/40
Qdot 705 nanocrystal	705	690LP	710/50	670LP	705/70
Qdot 800 nanocrystal	800	750LP	780/60	750LP	780/60

* Chattopadhyah, P. K., et al. (2006) Nature Med. 12:972

Target	Clone	Qdot 605	Qdot 655	Qdot 705	Qdot 800
Human					
CD3	S4.1		x		
CD3	UCHT1	x			
CD4	S3.5	x	x	x	
CD8	3B5	x	x	x	
CD10	MEM-78	Soon	Soon		
CD14	TüK4	x	x		x
CD27	CLB-27/1	x	x		
CD38	HIT2	x	x		
CD45	HI30	x	Soon	x	Soon
CD45RA	MEM-56	x	x		
Murine					
CD3	145-2C11	x			
CD4	RM4-8	x			