

Using Qdot® nanocrystal primary antibody conjugates in flow cytometry

TIPS FOR SAMPLE PREPARATION AND INSTRUMENT SETUP WHEN DETECTING SURFACE ANTIGENS.

Researchers are continually seeking to maximize the information that they receive from flow cytometry experiments by evaluating more labeled parameters in each sample. Qdot® nanocrystal conjugates are increasingly used in multispectral flow cytometry.¹⁻⁵ They provide a powerful way to multiply fluorophore selection using commonly available excitation sources. Invitrogen currently offers a growing selection of antibody conjugates using Qdot® 605, Qdot® 655, Qdot® 705, and Qdot® 800 nanocrystals. There are several unique advantages to using Qdot® nanocrystals, and here we will focus on their properties and use with common reagents and instrumentation in the flow cytometry workflow.

Qdot® nanocrystal technology is ideal for use in flow cytometry

Typical fluorescent dyes have excitation and emission spectra with relatively small Stokes shifts, which means that the optimal excitation wavelength is close to the emission peak. Qdot® nanocrystals have broad absorption spectra that increase dramatically at shorter excitation wavelengths. Their emission peaks are narrow and symmetrical and do not change with variations in the excitation source (Figure 1). Qdot® nanocrystals are desirable in that they require minimal single-laser compensation when using a single excitation source. Qdot® nanocrystals are optimally excited by a UV or violet (405–407 nm) laser, although sufficient excitation can also be obtained with other sources as discussed below.

The use of nanocrystal conjugates allows the addition of one to six colors, all excited from the violet laser, to panels using existing organic dyes. Qdot® nanocrystals provide the additional advantages of brightness and photostability.

Using Qdot® nanocrystal conjugates for surface antigens

Qdot® nanocrystal conjugates may be used in the same way as conventional conjugates. Conjugates are provided at a specific concentration

of Qdot® nanocrystal, usually 1–2 μM , and this concentration can be used to standardize experiments. Because staining conditions may vary, reagents should be titrated with samples to obtain optimal staining concentrations. Figure 2 shows typical staining patterns for a number of Qdot® antibody conjugates.

Reagents for sample preparation

Most conventional reagents used for erythrocyte lysis, including ammonium chloride and Cal-Lyse™ reagent, have minimal effects on the fluorescence intensity of cells stained with Qdot® conjugates (Figure 3). FACS™ Lysing Solution (BD Biosciences) usually has minimal impact on Qdot® nanocrystal fluorescence, although we have reports of occasional decreases in Qdot® nanocrystal fluorescence that may be related to particular batches of FACS™ Lysing Solution. In some cases, a decrease in fluorescence can be related to fixatives present in a lysis reagent such as BD™ PhosFlow Lyse (BD Biosciences), which may alter the antigenic determinants recognized by particular antibodies. →

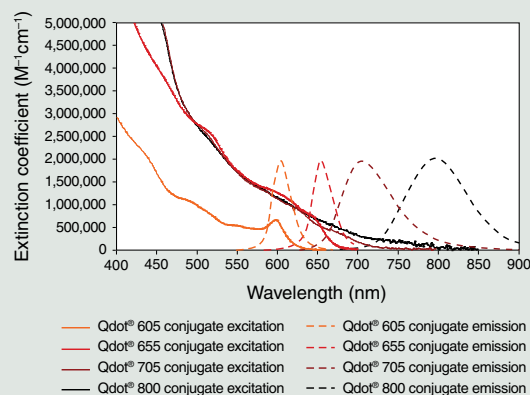


Figure 1—Extinction coefficient and emission profiles for selected Qdot® nanocrystals. Excitation is presented as extinction coefficient (left axis); emission is normalized to maximum peak height.

PRACTICAL APPLICATIONS

Table 1—BD™ LSR II filter combinations to detect selected Qdot® nanocrystals.

Fluorophore	Em*	Configuration A: Narrow bandpass filters		Configuration B: Broad bandpass filters ^{1,2}	
Qdot® 605 nanocrystal	605	570LP	605/20 nm	595LP	605/40 nm
Qdot® 655 nanocrystal	655	640LP	655/20 nm	640LP	660/40 nm
Qdot® 705 nanocrystal	705	690LP	720/20 nm	670LP	705/70 nm
Qdot® 800 nanocrystal	800	750LP	780/60 nm	750LP	780/60 nm

* Emission maximum, in nm.

Aldehyde-based fixatives may cause a small change in Qdot® nanocrystal fluorescence. For example, Figure 3 shows a 2-fold reduction in fluorescence after fixation with formaldehyde, although negative peak fluorescence also decreased. This change in fluorescence is generally tolerable given the population resolution achieved with Qdot® conjugates.

Reagents commonly used to permeabilize cells after fixation have not been shown to adversely affect Qdot® conjugate fluorescence. Reagents tested include FIX & PERM® Reagent B, BD Cytofix/Cytoperm™ reagent (BD Biosciences), 0.1% saponin, 0.05% Triton X®-100, and methanol solutions (Figure 3).

Instrument setup and filter selection

Qdot® nanocrystals exhibit the brightest emission when excited with either a UV or a violet laser source, but acceptable fluorescence can be obtained from any excitation below the emission maximum of a given nanocrystal. Therefore, samples stained with Qdot® nanocrystal conjugates can be analyzed on any cytometer that has an appropriate filter selection.

Because most nanocrystals have symmetrical and relatively narrow emission peaks (Figure 1), emission can be efficiently detected with a 20 nm wide filter centered on the emission maximum of a given nanocrystal. Users can minimize the need to correct for spectral overlap between Qdot® nanocrystals by selecting reagents with at least a 40 nm separation between maximum emissions. Table 1 shows two filter schemes that can be used with Qdot® nanocrystals on a BD™ LSR II cytometer. Configuration A uses narrow bandwidth filters to minimize effects of spectral overlap. Configuration B, developed in the laboratory of Mario Roederer, uses wider bandpass filters to collect more photons, and long-pass filters close the emission maximum to minimize spectral overlap.

Nanocrystals can also be used efficiently on instruments that have 488 nm excitation sources. For instruments with fixed filter configurations, such as the BD™ FACScan™ cytometer (BD Biosciences), you can match specific nanocrystals to the filters installed on the instrument.

Extend your color palette with Qdot® nanocrystals

Qdot® nanocrystal conjugates of monoclonal antibodies are powerful and easy-to-use tools to extend the number of colors in your multicolor flow cytometry panels. They are compatible with standard sample preparation reagents and staining protocols. They can be used efficiently on cytometers with UV or violet excitation sources, and with selection of appropriate filters. As with other fluorescent 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 if the nanocrystals are matched to available emission filters. For more information, visit www.invitrogen.com/qdotinflow. ■

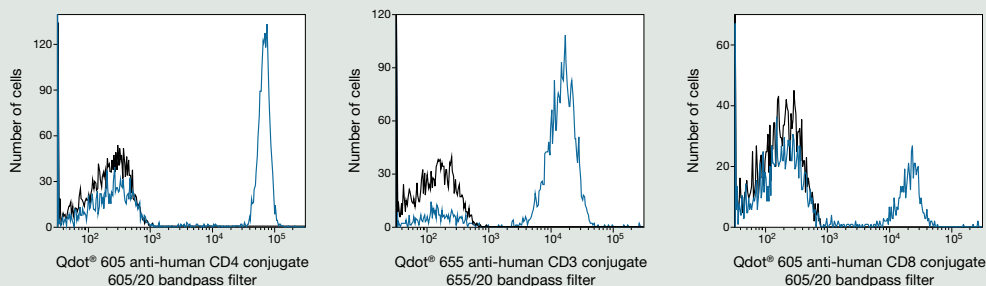


Figure 2—Staining profiles for Qdot® nanocrystal-conjugated antibodies. Human peripheral blood lymphocytes were stained with the specified antibody–Qdot® conjugates. Samples were analyzed using a BD™ LSR II cytometer (BD Biosciences) with 405 nm excitation and the specified emission filters. The blue peaks correspond to stained lymphocytes, and the black peaks show the position of unstained cells in the histograms.

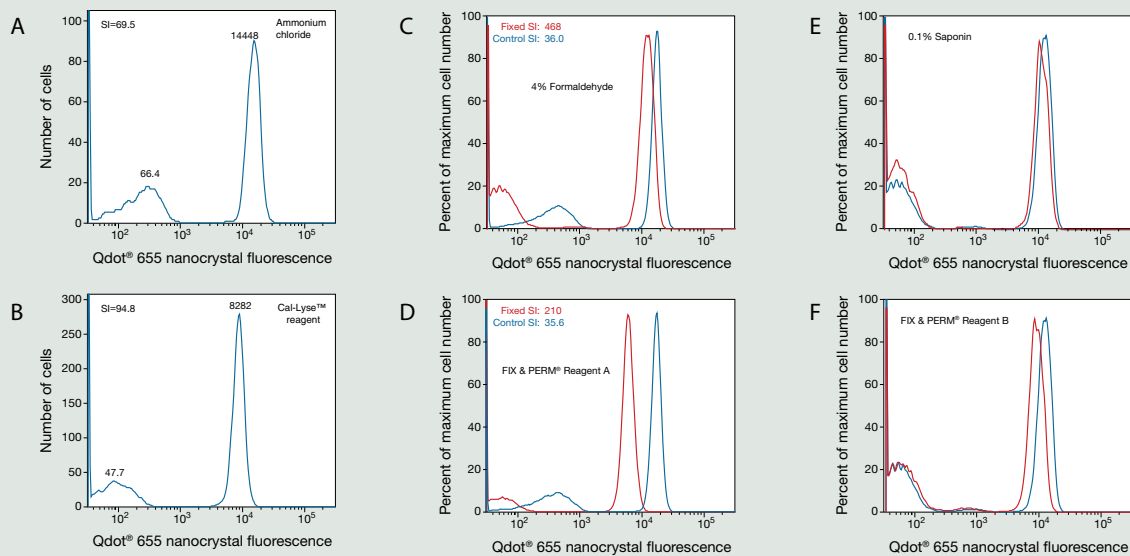


Figure 3—Representative results using common sample preparation reagents. Human peripheral blood lymphocytes were stained with mouse anti-human CD4, Qdot® 655 conjugate, before treatment with the specified reagents: erythrocyte lysis (A, B), fixatives (C, D) and permeabilizing reagents after treatment with formaldehyde (E, F). Samples were analyzed using a BD™ LSR II cytometer (BD Biosciences) with 405 nm excitation and a 655/20 nm emission filter. Histograms are smoothed and labeled with geometric mean fluorescence values. Staining index (SI), to quantify population resolution, is calculated as the difference in population mean fluorescence values divided by twice the negative peak standard deviation.

References

1. Chattopadhyay, P.K. et al. (2006) *Nat Med* 12:972–977.
2. Perfetto, S.P. et al. (2004) *Nat Rev Immunol* 4:648–655.
3. Telford, W.G. (2004) *Cytometry A* 61:9–17.
4. Chattopadhyay, P.K. et al. (2007) in *Quantum Dots, Applications in Biology*. M.P. Bruchez and C.Z. Hotz, ed. Humana Press, Totowa, NJ. pp. 175–184.
5. Abrams, B. and Dubrovsky, T. (2007) in *Quantum Dots, Applications in Biology*. Bruchez, M.P. and Hotz, C.Z., ed. Humana Press, Totowa, NJ. pp. 185–206.

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Product	Quantity	Cat. no.	Product	Quantity	Cat. no.
CD3, mouse anti-human, Qdot® 605 conjugate	100 µl	Q10054	CD45RA, mouse anti-human, Qdot® 605 conjugate	100 µl	Q10047
CD3, mouse anti-human, Qdot® 655 conjugate	100 µl	Q10012	CD45RA, mouse anti-human, Qdot® 655 conjugate	100 µl	Q10069
CD4, mouse anti-human, Qdot® 605 conjugate	100 µl	Q10008	Mouse IgG2a, Qdot® 605 conjugate	100 µl	Q10014
CD4, mouse anti-human, Qdot® 655 conjugate	100 µl	Q10007	Mouse IgG2a, Qdot® 655 conjugate	100 µl	Q10015
CD4, mouse anti-human, Qdot® 800 conjugate	100 µl	Q10064	Mouse IgG2a, Qdot® 705 conjugate	100 µl	Q10076
CD8, mouse anti-human, Qdot® 605 conjugate	100 µl	Q10009	Mouse IgG2a, Qdot® 800 conjugate	100 µl	Q10075
CD8, mouse anti-human, Qdot® 655 conjugate	100 µl	Q10055	Mouse IgG2b, Qdot® 605 conjugate	100 µl	Q10074
CD8, mouse anti-human, Qdot® 705 conjugate	100 µl	Q10059	Mouse IgG1, Qdot® 605 conjugate	100 µl	Q10073
CD14, mouse anti-human, Qdot® 605 conjugate	100 µl	Q10013	Cal-Lyse™ Whole Blood Lysing Solution	25 ml	GAS-010
CD14, mouse anti-human, Qdot® 655 conjugate	100 µl	Q10056	Cal-Lyse™ Whole Blood Lysing Solution	100 ml	GAS-010S-100
CD14, mouse anti-human, Qdot® 800 conjugate	100 µl	Q10064	High-Yield Lyse	500 ml	HYL-250
CD27, mouse anti-human, Qdot® 605 conjugate	100 µl	Q10065	FIX & PERM® Reagent A	5 ml	GAS001S-5
CD27, mouse anti-human, Qdot® 655 conjugate	100 µl	Q10066	FIX & PERM® Reagent A	100 ml	GAS001S-100
CD38, mouse anti-human, Qdot® 605 conjugate	100 µl	Q10053	FIX & PERM® Reagent B	5 ml	GAS002S-5
CD38, mouse anti-human, Qdot® 655 conjugate	100 µl	Q10057	FIX & PERM® Reagent B	100 ml	GAS002S-100
CD45, mouse anti-human, Qdot® 605 conjugate	100 µl	Q10051	FIX & PERM® Reagents	50 ml	GAS003
CD45, mouse anti-human, Qdot® 705 conjugate	100 µl	Q10062	FIX & PERM® Reagents	200 ml	GAS004