

## Qdot® Anti-Human CD3 Antibody Conjugates

Product	Form	Volume	Qdot®	Tests	Peak		Recommended
			Nanocrystal Conc.		Excitation (nm)	Emission (nm)	
Q10054	Qdot® 605	0.1 mL	1 µM	100 min.	405 (488)*	605	605/20
<b>Isotype Control: Mouse IgG1</b>							
Q10073	Qdot® 605	0.1 mL	1 µM	100 min.	405 (488)*	605	605/20

\*Qdot® nanocrystals excite optimally in the UV to 405 nm range, but can also be excited with wavelengths shorter than their emission maximum, such as with a 488 nm laser.

### Product Description

Mouse monoclonal antibody to the human CD3 antigen. Suitable for intracellular staining protocols.

**Clone:** UCHT1

**Isotype:** Mouse IgG1

**Lot No.:** See label **Expiration:** See label

**Buffer:** 50 mM borate, 1 M betaine, pH 8.3

**Preservative:** 0.05% sodium azide. Sodium azide is an extremely toxic and dangerous compound particularly when combined with acids or metals. Properly dispose of solutions containing sodium azide.

### Storage and Handling

Store reagents at 2–8°C. **Do not freeze.** Because Qdot® nanocrystals are conjugated to biological materials, some loss of activity may be observed with prolonged storage.

Qdot® nanocrystals are photostable, and do not need to be protected from light. However, if using Qdot® conjugates in combination with conventional fluorochrome conjugated antibodies, minimize light exposure during handling, incubation with cells, and prior to analysis. We recommend analysis of cells within 18 hours of staining. If dilute reagent is used, dilute only the quantity of reagent to be used within one day.

The Qdot® conjugate contains cadmium and selenium in an inorganic crystalline form. 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.

### Qdot® Nanocrystals

Qdot® nanocrystals are nanometer-scale atom clusters of semiconductor material which exhibit narrow and symmetrical emission bandwidths with very long Stokes shifts. The nanocrystals can be excited with any wavelength below their emission maximum, but are best excited by UV or violet light. Qdot® nanocrystals demonstrate an intrinsic brightness and photostability that can be many times greater than observed with other classes of fluorophores. These advantages make Qdot® nanocrystals powerful tools for antibody staining.<sup>1-2</sup>

### Product Characterization

**Antigen Specificity:** The UCHT1 monoclonal antibody reacts with human CD3e, a 20 kDa subunit of the TCR/CD3 complex, even after aldehyde fixation.<sup>3-5</sup> Along with the other CD3 subunits  $\gamma$  and  $\delta$ , the  $\epsilon$  chain is required for proper assembly, trafficking and surface expression of the TCR complex. CD3 is expressed by developing thymocytes and by all mature T cells. Crosslinking of TCR via immobilized UCHT1 results in T cell proliferation.

**Leukocyte Workshop Status:** Leukocyte Typing III, IV and V

### Product Use

**Staining:** Stain cells in any standard staining buffer, such as phosphate-buffered saline (PBS) with 1% bovine serum albumin (BSA). Use 1 µL of Qdot® antibody conjugate per  $1 \times 10^6$  cells in a 100 µL staining volume (10 nM final concentration of Qdot® nanocrystals). Qdot® nanocrystal conjugates may be mixed with other antibodies, but use the diluted conjugates on the day of dilution. Because conditions may vary, you may need to determine the optimal amount of antibody used for each

application. Qdot® nanocrystal conjugates can be used for surface staining applications with most conventional sample preparation reagents, such as Caltag® Cal-Lyse™ and Caltag® FIX & PERM® reagents, with minimal affect on fluorescence. We have observed that some batches of BD FACS™ Lysing Solution have been found to interfere with Qdot® nanocrystal fluorescence.

**Instrument setup:** Qdot® nanocrystals are excited optimally with UV or 405 nm light, although excitation can be obtained with any wavelength below the emission wavelength of a given nanocrystal. Make sure the cytometer has an appropriate emission filter for the Qdot® nanocrystal being used. The table above has filter recommendations; alternate filters can be used as long as they capture the emission maximum, but filter width impacts spectral overlap corrections. **Note:** Qdot® nanocrystals can be used on cytometers that do not have UV or violet excitation sources as long as they have appropriate emission filters. Be sure to check for Qdot® nanocrystal emission in any channel that can capture nanocrystal emission off of other lasers on the cytometer.

### Product Quality Control

Each lot has been tested by flow cytometry using human peripheral blood leukocytes (PBLs). This testing was performed using 1 µL of antibody per  $1 \times 10^6$  cells in a 100 µL staining volume (10 nM final concentration of Qdot® nanocrystals). Qdot® nanocrystal concentration is assigned based on optical density. See reverse for representative flow cytometry data.

### References

- Telford, W. G. 2004. *Cytometry Part A* 61A:9.
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- Schlossman, S. F., L. Boumsell, W. Gilks, J. M. Harlan, T. Kishimoto, C. Morimoto, J. Ritz, S. Shaw, R. Silverstein, T. Springer, T. F. Tedder, and R. F. Todd eds. 1995. *Leukocyte Typing V*. Oxford University Press Inc., New York.
- Garson, J. A., P. C. Beverley, H. B. Coakham, and E. I. Harper. 1982. *Nature* 298(5872):375-377.

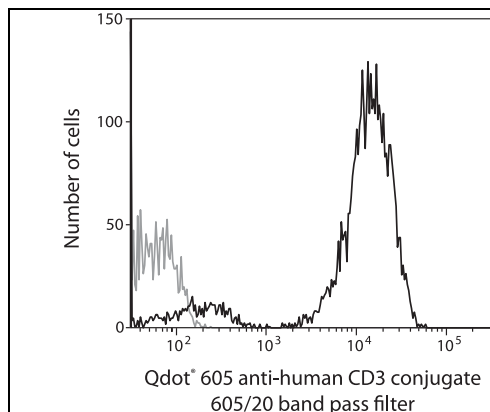
### Related Products

Catalog no.	Product Name	Unit Size
GAS-010	Cal-Lyse™ Whole Blood Lysing Solution	25 mL
GAS-010S-100	Cal-Lyse™ Whole Blood Lysing Solution	100 mL
HYL-250	High-Yield Lyse Fixative	500 mL
GAS001S-5	FIX & PERM® Reagent A (Individual)	5 mL
GAS001S-100	FIX & PERM® Reagent A (Bulk)	100 mL
GAS002S-5	FIX & PERM® Reagent B (Individual)	5 mL
GAS002S-100	FIX & PERM® Reagent B (Bulk)	100 mL
GAS-003	FIX & PERM® Reagents	50 tests
GAS-004	FIX & PERM® Reagents	200 tests

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Log fluorescence intensity profile of human PBLs gated on lymphocytes and analyzed on a BD LSR II flow cytometer (BD Biosciences, San Jose, CA) using 405 nm excitation and the specified emission filters. The data files were analyzed using FlowJo™ software (Treestar, Inc., [www.flowjo.com](http://www.flowjo.com)). The black line represents cells stained with anti-human CD3 antibody conjugate and the gray line represents unstained cells.

**Note:** Flow cytometric data shown may not necessarily have been generated using the enclosed lot of reagent. For this reason, and due to differences in flow cytometers and cytometer settings, results may vary from those illustrated above. We recommend titrating reagents to determine optimal conditions for use in your systems.

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