

Qdot® Anti-Mouse CD3 Antibody Conjugate

Product	Form	Volume	Qdot®	Tests	Peak		Recommended
			Nanocrystal Conc.		Excitation (nm)	Emission (nm)	
Q10090	Qdot® 605	0.1 mL	1 µM	100 min.	405 (488)*	605	605/20
Isotype Control: Hamster IgG							
Q10159	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

Hamster monoclonal antibody to the mouse CD3 antigen.

Clone: 145-2C11

Isotype: Hamster IgG

Immunogen: H-2K^b-specific murine CTL clone (BM10-37)¹

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.²⁻³

Product Characterization

Antigen Specificity: The 145-2C11 monoclonal antibody (mAb) is specific for the 25 kDa chain of mouse CD3, which is a component of the multimeric T cell receptor complex involved in signal transduction upon MHC-antigen recognition.¹ In mouse, the CD3 is highly expressed by mature T cells and is expressed at lower levels by CD4⁺CD8⁺ thymocytes. In addition to immunostaining for flow cytometry,^{1,4,6} other reported applications for the 145-2C11 mAb include immunoprecipitation,^{1,5} western blot analysis,⁶ immunohistochemistry on acetone-fixed frozen sections,⁷ and functional assays.¹

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 × 10⁶ 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 for use with each application. Qdot® nanocrystal conjugates can be used for surface staining

applications with most conventional sample preparation reagents, such as Cal-Lyse™ and FIX & PERM® reagents, with minimal affect on fluorescence. Also, as the 145-2C11 mAb is known to exhibit Fc receptor-mediated binding to mouse cells, the use of purified anti-mouse CD16/32 antibody (Invitrogen Cat. no. MFCR00) to reduce this effect is strongly encouraged. We have observed that some batches of BD FACS™ Lysing Solution 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

Every lot is tested by flow cytometry using freshly harvested mouse splenocytes. The testing is performed using 1 µL of antibody conjugate per 1 × 10⁶ 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

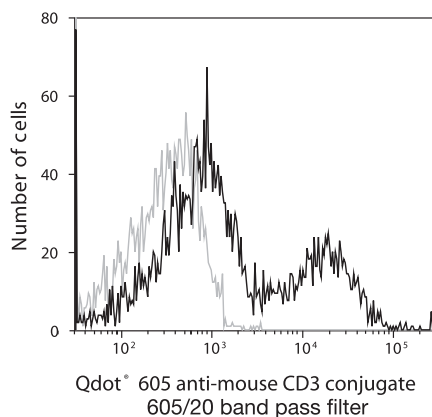
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2. Telford, W. G. 2004. *Cytometry Part A* 61A:9.
3. Perfetto, S. P., P. K. Chattopadhyah, and M. Roederer. 2004. *Nature Reviews - Immunology* 4: 648.
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Related Products

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 mouse splenocytes 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). The black line represents cells stained with anti-mouse 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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