

## Qdot® Anti-Mouse CD45R (B220) Antibody Conjugates

Product	Form	Volume	Qdot®	Tests	Peak		Recommended
			Nanocrystal Conc.		Excitation (nm)	Emission (nm)	
Q10176	Qdot® 655	0.1 mL	1 µM	100 min	405 (488)*	655	655/20
<b>Isotype Control: Mouse IgG2a</b>							
Q10158	Qdot® 655	0.1 mL	1 µM	100 min	405 (488)*	655	655/20

\*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

Rat monoclonal antibody to the mouse CD45R (B220) antigen

**Clone:** RA3-6B2

**Isotype:** Rat IgG2a

**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 using dilute reagent, 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 RA3-6B2 monoclonal antibody (mAb) reacts with an exon A-specific epitope on CD45 that is expressed on essentially all B cells and is maintained throughout B cell development<sup>3,4</sup>. The RA3-6B2 epitope is expressed in a restricted manner by B cells; however, it is also present on lymphokine activated killer (LAK) cells<sup>1</sup>. Although B220 has been widely used as a pan B cell marker, CD19 may be a more appropriate pan B cell marker, as expression of CD19 appears to be more closely restricted to B cells. The RA3-6B2 mAb has been shown to potentiate isotype switching in B cells and to inhibit proliferative responses of B cell mitogens on B cells<sup>5,6</sup>. Applications of RA3-6B2 include immunostaining for flow cytometry and immunoprecipitation<sup>1</sup>.

**Leukocyte Workshop Status:** N/A

### 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 for use with 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 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 your 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 mouse splenocytes. 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

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- Perfetto, S. P., P. K. Chattopadhyah, and M. Roederer. 2004. *Nature Reviews - Immunology* 4: 648.
- Coffman, R. L. 1982. Surface antigen expression and immunoglobulin gene rearrangement during mouse pre-B cell development. *Immunol. Rev.* 69: 5–23.
- Johnson, P., A. Maiti, and D. H. W. Ng. 1997. CD45: A family of leukocyte-specific cells surface glycoproteins. In *Wier's Handbook of Experimental Immunology*, Vol. 2. L. A. Herzenberg, D. M. Weir, and C. Blackwell, eds. Blackwell Science, Cambridge, MA, pp.62.1–62.16.
- George, A., S. Rath, K. E. Shroff, M. Wang, and J. M. Durdik. 1994. Ligation of CD45 on B cells can facilitate production of secondary Ig isotypes. *J. Immunol.* 152: 1014–1021.
- Domati-Saad, R., E. W. Ogle, and L. B. Justement. 1993. Administration of anti-CD45 mAb specific for a B cell-restricted epitope abrogates the B cell response to a T-dependent antigen *in vivo*. *J. Immunol.* 151: 5936–5947

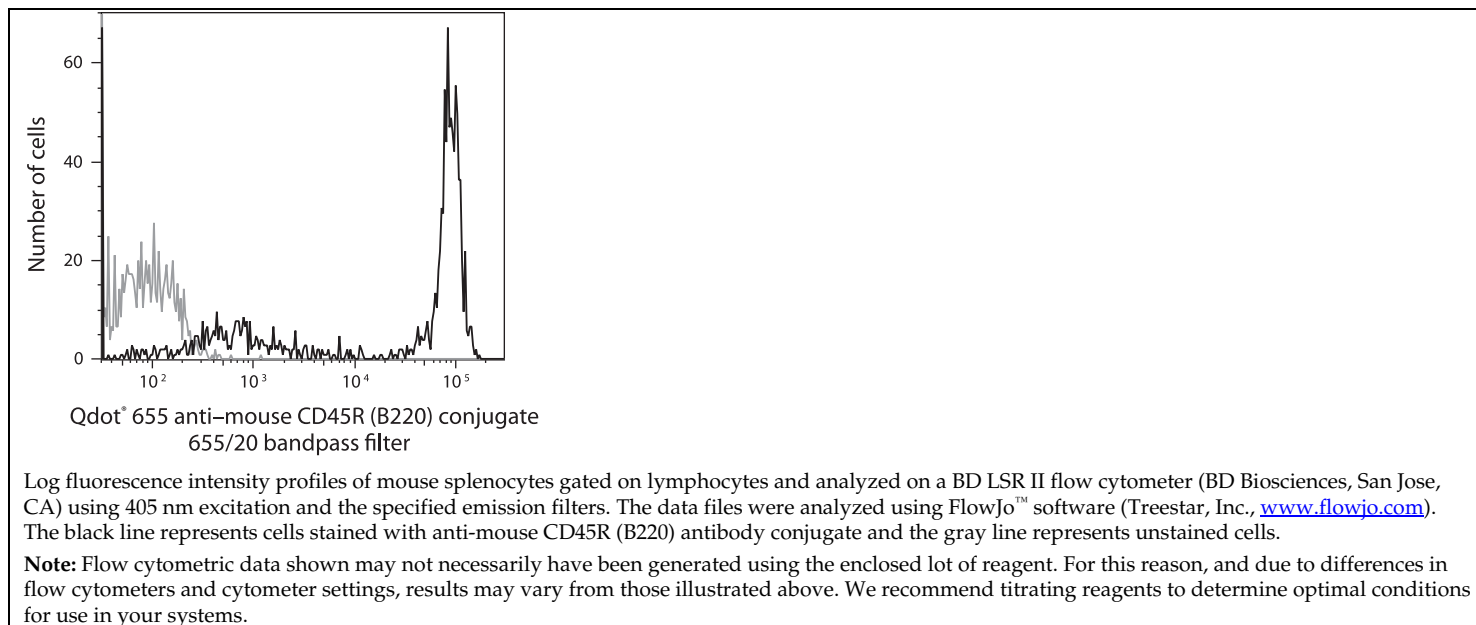
### Related Products

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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