Technical Data Sheet

PerCP-Cy™5.5 Rat Anti-Mouse CD45R/B220

Product Information

Material Number:	561101
Alternate Name:	B220; Ly-5; CD45R; LCA; Ptprc; Protein tyrosine phosphatase receptor type C
Size:	25 μg
Concentration:	0.2 mg/ml
Clone:	RA3-6B2
Immunogen:	Mouse Abelson Leukemia Virus-Induced pre-B tumor cells
Isotype:	Rat IgG2a, ĸ
Reactivity:	QC Testing: Mouse
	Tested in Development: Human
Storage Buffer:	Aqueous buffered solution containing $\leq 0.09\%$ sodium azide.

Description

The rat anti-mouse CD45R antibody (clone RA3-6B2) has been reported to react with an epitope on the extracellular domain of the transmembrane CD45 glycoprotein which is dependent upon the expression of exon A and specific carbohydrate residues. It is expressed on B lymphocytes at all stages from pro-B through mature and activated B cell, but it is decreased on plasma cells and a subset of memory B cells. The levels of CD45R expression on the B-cell lineage appear to be developmentally regulated. It is also reportedly found on the abnormal T cells involved in the lymphadenopathy of *lpr/lpr* and *gld/gld* mutant mice, on lytically active subsets of lymphokine-activated killer cells (NK cells and non-MHC-restricted CTL), on apoptotic T lymphocytes, and macrophage progenitors in fetal liver. The CD45R antigen has been reported not to be on hematopoietic stem cells, naive T lymphocytes, or MHC-restricted CTL. CD45 is a member of the Protein Tyrosine Phosphatase (PTP) family: Its intracellular (COOH-terminal) region contains two PTP catalytic domains, and the extracellular region is highly variable due to alternative splicing of exons 4, 5, and 6 (designated A, B, and C, respectively), plus differing levels of glycosylation. The CD45 isoforms detected in the mouse are cell type-, maturation, and activation state-specific. The CD45 isoforms play complex roles in T-cell and B-cell antigen receptor signal transduction. CD45R is commonly used as a pan B-cell marker; however, CD19 expression, detectable by the rat anti-mouse CD19 antibody (clone 1D3), is reported to be more restricted to the B-cell lineage. The rat anti-mouse CD45R antibody (clone RA3-6B2) has been reported to enhance isotype switching during *in vitro* B-cell responses and to inhibit *in vivo* B-cell responses. Cross-reaction of the RA3-6B2 clone with activated human T lymphocytes has also been reportedly observed.

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography. The antibody was conjugated with PerCP-Cy5.5 under optimum conditions, and unconjugated antibody and free PerCP-Cy5.5 were removed. Storage of PerCP-Cy5.5 conjugates in unoptimized diluent is not recommended and may result in loss of signal intensity. Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application Routinely Tested Flow cytometry Routinely Tested Suggested Companion Products Size Clone Stalog Number PerCP-Cy™5.5 Rat IgG2a, κ Isotype Control 0.1 mg R35-95

Product Notices

- 1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
- 2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
- PerCP-Cy5.5–labelled antibodies can be used with FITC- and R-PE–labelled reagents in single-laser flow cytometers with no significant spectral overlap of PerCP-Cy5.5, FITC, and R-PE fluorescence.
- 4. PerCP-Cy5.5 is optimized for use with a single argon ion laser emitting 488-nm light. Because of the broad absorption spectrum of the tandem fluorochrome, extra care must be taken when using dual-laser cytometers, which may directly excite both PerCP and Cy5.5™. We recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.
- 5. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
- 6. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.

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- 7. PerCP is a photosynthetic accessory pigment from Glenodinium species of dinoflagellates, which is excited by the 488-nm light of an Argon ion laser and fluoresces at 675 nm. Therefore, PerCP-labelled antibodies can be used with FITC- and R-PE–labelled reagents in most single-laser flow cytometers with no significant spectral overlap of PerCP fluorescence with that of FITC or R-PE. PerCP has been reported to undergo significant photobleaching, the magnitude of which increases as laser power is increased or beam focus is narrowed. For third-color flow¬cytometric analysis using ≥25-mW laser power, we recommend PE-Cy5-, PE-Cy7–, or PerCP-Cy5.5-conjugated reagents.
- 8. Cy is a trademark of Amersham Biosciences Limited. This conjugated product is sold under license to the following patents: US Patent Nos. 5,486,616; 5,569,587; 5,569,766; 5,627,027.
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References

Bleesing JJ, Morrow MR, Uzel G, Fleisher TA. Human T cell activation induces the expression of a novel CD45 isoform that is analogous to murine B220 and is associated with altered O-glycan synthesis and onset of apoptosis. *Cell Immunol.* 2001; 213(1):72-81. (Biology) Coffman RL. Surface antigen expression and immunoglobulin gene rearrangement during mouse pre-B cell development. *Immunol Rev.* 1982; 69:5-23.

(Immunogen) Driver DJ, McHeyzer-Williams LJ, Cool M, Stetson DB, McHeyzer-Williams MG. Development and maintenance of a B220- memory B cell compartment. J Immunol. 2001; 167(3):1393-1405. (Biology)