

Technical Data Sheet

V500 Rat anti-Mouse CD45R/B220

Product Information

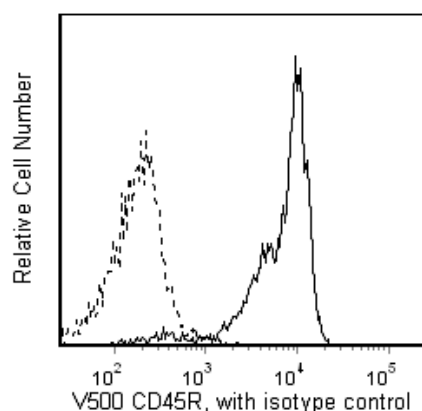
Material Number:	561227
Alternate Name:	B220; Ly-5; CD45R; LCA; Ptprc; Protein tyrosine phosphatase receptor type C
Size:	25 µg
Concentration:	0.2 mg/ml
Clone:	RA3-6B2
Immunogen:	Mouse Abelson Leukemia Virus-Induced pre-B tumor cells
Isotype:	Rat IgG2a, κ
Reactivity:	QC Testing: Mouse; Reported: Human
Storage Buffer:	Aqueous buffered solution containing protein stabilizer, glycerol and ≤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 of mice injected with bacterial superantigen, on a population of NK-cell precursors in the bone marrow, and on B-lymphocyte, T-lymphocyte, 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.

The antibody is conjugated to BD Horizon™ V500, which has been developed for use in multicolor flow cytometry experiments and is available exclusively from BD Biosciences. It is excited by the Violet laser with an Ex max of 415 nm and Em Max at 500 nm. BD Horizon V500 conjugates emit at a similar wavelength to Amcyan yet exhibit reduced spillover into the FITC channel. For more information on BD Horizon V500, visit bdbiosciences.com/colors.

When compensating dyes in this spectral range (such as Horizon™ V500 and AmCyan), the most accurate compensation can be obtained using single stained cellular controls. Due to spectral differences between cells and beads in this channel, using BD CompBeads can result in spillover errors for V500 and AmCyan reagents. Therefore, the use of BD CompBeads or BD CompBeads Plus to determine spillover values for these reagents is not recommended. Different V500 reagents (e.g. CD4 vs. CD45) can have slightly different fluorescence spillover therefore, it may also be necessary to use clone specific compensation controls when using these reagents.

**Analysis of CD45R expression on mouse splenocytes.**

Splenocytes from BALB/c mice were stained simultaneously with BD Horizon™ V500 Rat anti-Mouse CD45R/B220 (Cat. No. 561227; solid line histogram) or BD Horizon™ V500 Rat IgG2a, κ Isotype Control (Cat. No. 560786 used at the same concentration, dashed line histogram), and PE Hamster Anti-Mouse CD3e (Cat. No. 553063/553064). The fluorescence histograms were derived from CD3-negative gated events with the forward and side light-scatter characteristics of viable lymphocytes. Flow cytometry was performed using a BD FACSCanto™ II Flow Cytometer System.

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Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated with BD Horizon™ V500 under optimum conditions, and unreacted BD Horizon™ V500 was removed.

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Flow cytometry	Routinely Tested
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Suggested Companion Products

Catalog Number	Name	Size	Clone
560786	V500 Rat IgG2a, κ Isotype Control	0.1 mg	R35-95
553064	PE Hamster Anti-Mouse CD3e	0.2 mg	145-2C11
553063	PE Hamster Anti-Mouse CD3e	0.1 mg	145-2C11
554656	Stain Buffer (FBS)	500 ml	(none)

Product Notices

1. Please refer to www.bdbiosciences.com/pharming/protocols for technical protocols.
2. BD Horizon™ V500 has a maximum absorption of 415 nm and maximum emission of 500 nm. Before staining with this reagent, please confirm that your flow cytometer is capable of exciting the fluorochrome and discriminating the resulting fluorescence.
3. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
4. 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.

References

Allman DM, Ferguson SE, Cancro MP. Peripheral B cell maturation. I. Immature peripheral B cells in adults are heat-stable antigenhi and exhibit unique signaling characteristics. *J Immunol.* 1992; 149(8):2533-2540. (Biology)

Asensi V, Kimeno K, Kawamura I, Sakumoto M, Nomoto K. Treatment of autoimmune MRL/lpr mice with anti-B220 monoclonal antibody reduces the level of anti-DNA antibodies and lymphadenopathies. *Immunology.* 1989; 68(2):204-208. (Clone-specific: Inhibition)

Ballas ZK, Rasmussen W. Lymphokine-activated killer cells. VII. IL-4 induces an NK1.1+CD8 alpha+beta- TCR-alpha beta B220+ lymphokine-activated killer subset. *J Immunol.* 1993; 150(1):17-30. (Biology)

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. (Clone-specific)

Coffman RL. Surface antigen expression and immunoglobulin gene rearrangement during mouse pre-B cell development. *Immunol Rev.* 1982; 69:5-23. (Immunogen: Immunoprecipitation)

Domati-Saad R, Ogle EW, Justement LB. 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.* 1993; 151(11):5936-5947. (Clone-specific: Inhibition)

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)

George A, Rath S, Shroff KE, Wang M, Durdik JM. Ligation of CD45 on B cells can facilitate production of secondary Ig isotypes. *J Immunol.* 1994; 152(3):1014-1021. (Clone-specific: (Co)-stimulation, Stimulation)

Hardy RR, Carmack CE, Shinton SA, Kemp JD, Hayakawa K. Resolution and characterization of pro-B and pre-pro-B cell stages in normal mouse bone marrow. *J Exp Med.* 1991; 173(5):1213-1225. (Biology)

Hathcock KS, Hirano H, Murakami S, Hodes RJ. CD45 expression by B cells. Expression of different CD45 isoforms by subpopulations of activated B cells. *J Immunol.* 1992; 149(7):2286-2294. (Biology)

Johnson P, Maiti A. CD45: A family of leukocyte-specific cell surface glycoproteins. In: Herzenberg LA, Weir DM, Blackwell C, ed. *Weir's Handbook of Experimental Immunology, Vol 2.* Cambridge: Blackwell Science; 1997:62.1-62.16. (Biology)

Kobata T, Takasaki K, Asahara H, et al. Apoptosis with FasL+ cell infiltration in the periphery and thymus of corrected autoimmune mice. *Immunology.* 1997; 92(2):206-213. (Biology)

Krop I, de Fougerolles AR, Hardy RR, Allison M, Schlissel MS, Fearon DT. Self-renewal of B-1 lymphocytes is dependent on CD19. *Eur J Immunol.* 1996; 26(1):238-242. (Biology)

Laouar Y, Ezine S. In vivo CD4+ lymph node T cells from lpr mice generate CD4-CD8-B220+TCR-beta low cells. *J Immunol.* 1994; 153(9):3948-3955. (Biology)

Puzanov IJ, Bennett M, Kumar V. IL-15 can substitute for the marrow microenvironment in the differentiation of natural killer cells. *J Immunol.* 1996; 157(10):4282-4285. (Biology)

Rolink A, ten Boekel E, Melchers F, Fearon DT, Krop I, Andersson J. A subpopulation of B220+ cells in murine bone marrow does not express CD19 and contains natural killer cell progenitors. *J Exp Med.* 1996; 183(1):187-194. (Biology)

Sagara S, Sugaya K, Tokoro Y, et al. B220 expression by T lymphoid progenitor cells in mouse fetal liver. *J Immunol.* 1997; 158(2):666-676. (Biology)