## **Technical Data Sheet**

# PE-CF594 Rat Anti-Mouse CD45R/B220

#### **Product Information**

Material Number: 562313

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

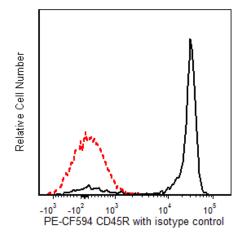
 $\begin{tabular}{lll} \textbf{Isotype:} & Rat IgG2a, \kappa \\ \textbf{Reactivity:} & QC Testing: Mouse \\ \end{tabular}$ 

Storage Buffer: Aqueous buffered solution containing BSA 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.

This antibody is conjugated to BD Horizon<sup>TM</sup> PE-CF594, which has been developed exclusively by BD Biosciences as a better alternative to PE-Texas Red. PE-CF594 excites and emits at similar wavelengths to PE-Texas Red yet exhibits improved brightness and spectral characteristics. Due to PE having maximal absorption peaks at 496 nm and 564 nm, PE-CF594 can be excited by the blue (488-nm), green (532-nm) and yellow-green (561-nm) lasers and can be detected with the same filter set as PE-Texas Red (eg 610/20-nm filter).



Flow cytometric analysis of CD45R/B220 expressed on mouse splenocytes. Splenocytes from a BALB/c mouse were stained with either BD Horizon™ PE-CF594 Rat IgG2a, κ Isotype Control (Cat. No. 562302; dashed line histogram) or BD Horizon™ PE-CF594 PE-CF594 Rat Anti-Mouse CD45R antibody (Cat. No. 562290/562313; solid line histogram). The fluorescence histograms were derived from events with the forward and side light-scatter characteristics of viable splenocytes. Flow cytometry was performed using a BD™ LSR II Flow Cytometer System.

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#### **Preparation and Storage**

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

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

The antibody was conjugated with BD Horizon™ PE-CF594 under optimum conditions, and unconjugated antibody and free PE-CF594 were removed.

### **Application Notes**

### Application

1		
	Routinely Tested	

## **Suggested Companion Products**

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 ml	(none)
562302	PE-CF594 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. When excited by the yellow-green (561-nm) laser, the fluorescence may be brighter than when excited by the blue (488-nm) laser.
- Because of the broad absorption spectrum of the tandem fluorochrome, extra care must be taken when using multi-laser cytometers, which
  may directly excite both PE and CFTM594.
- 4. An isotype control should be used at the same concentration as the antibody of interest.
- 5. Please observe the following precautions: Absorption of visible light can significantly alter the energy transfer occurring in any tandem fluorochrome conjugate; therefore, we recommend that special precautions be taken (such as wrapping vials, tubes, or racks in aluminum foil) to prevent exposure of conjugated reagents, including cells stained with those reagents, to room illumination.
- 6. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
- 7. CF<sup>TM</sup> is a trademark of Biotium, Inc.
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- 9. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
- 10. 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.
- 11. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

### 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)

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. (Biology) Domiati-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)

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)

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)

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)

Renno T, Hahne M, Tschopp J, MacDonald HR. Peripheral T cells undergoing superantigen-induced apoptosis in vivo express B220 and upregulate Fas and Fas ligand. *J Exp Med.* 1996; 183(2):431-437. (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)

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