Technical Data Sheet

BUV395 Rat Anti-Mouse CD45R/B220

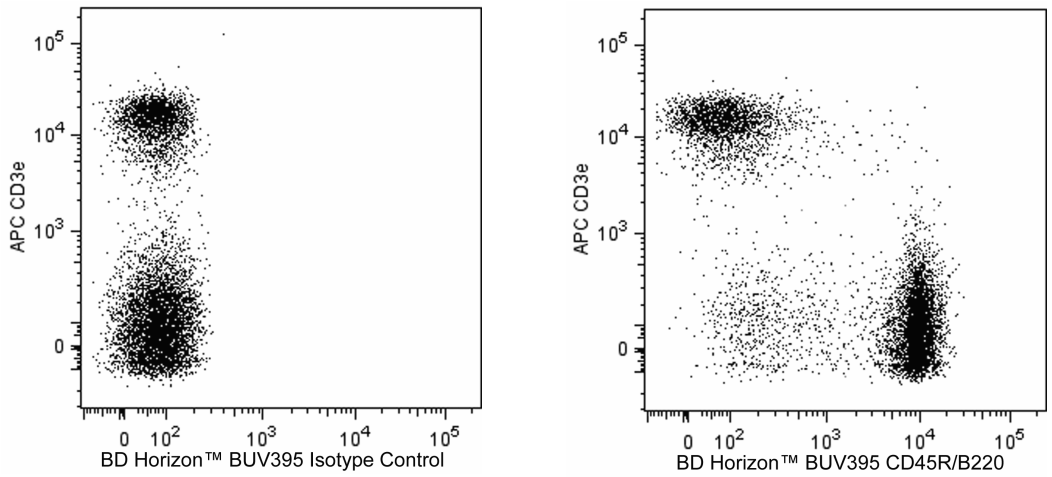
Product Information

Material Number:	**563793**
Alternate Name:	B220; Ly-5; CD45R; LCA; Ptprc; Protein tyrosine phosphatase receptor type C
Size:	50 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 Reactivity: Human
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.

The antibody was conjugated to BD Horizon™ BUV395 which has been exclusively developed by BD Biosciences as an optimal dye for use on a 355 nm laser equipped instrument. With an Ex Max at 348 nm and an Em Max at 395 nm, this dye has virtually no spillover into any other detector. BD Horizon™ BUV395 can be excited with a 355 nm laser and detected with a 379/28 filter.



Two-color flow cytometric analysis of CD45R/B220 expressed on mouse splenocytes. *Mouse splenic leucocytes were preincubated with Purified Rat Anti-Mouse CD16/CD32 antibody (Mouse BD Fc Block™) (Cat. No. 553141/553142). The cells were then stained with APC Hamster Anti-Mouse CD3e antibody (Cat. No. 553066/561826) and either BD Horizon™ BUV395 Rat IgG2a, κ Isotype Control (Cat. No. 563556; Left Panel) or BD Horizon™ BUV395 Rat Anti-Mouse CD45R/B220 antibody (Cat. No. 563793; Right Panel). Two-color flow cytometric dot plots show the correlated expression patterns of CD45R/B220 (or Ig Isotype control staining) versus CD3 for gated events with the forward and side light-scatter characteristics of viable splenic leucocytes. Flow cytometric analysis 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™ BUV395 under optimum conditions, and unconjugated antibody and free BD Horizon™ BUV395 were removed.

Application Notes

Application

Flow cytometry	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 ml	(none)
563556	BUV395 Rat IgG2a, κ Isotype Control	50 µg	R35-95
553066	APC Hamster Anti-Mouse CD3e	0.1 mg	145-2C11
561826	APC Hamster Anti-Mouse CD3e	25 µg	145-2C11
553141	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.1 mg	2.4G2
553142	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.5 mg	2.4G2
555899	Lysing Buffer	100 ml	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. An isotype control should be used at the same concentration as the antibody of interest.
3. 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.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
5. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.
6. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
7. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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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. (Clone-specific: Flow cytometry, Fluorescence activated cell sorting, Immunofluorescence)

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: Flow cytometry, Immunofluorescence)

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

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: In vivo exacerbation)

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. (Clone-specific: Flow cytometry, Fluorescence activated cell sorting, Fluorescence microscopy, Immunofluorescence)

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, Functional assay)

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. (Clone-specific: Flow cytometry, Fluorescence activated cell sorting, Immunofluorescence)

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. (Clone-specific: Flow cytometry)

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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. (Clone-specific: Flow cytometry, Fluorescence activated cell sorting, Immunofluorescence)

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