

Technical Data Sheet

BV421 Rat Anti-Mouse CD45R/B220

Product Information

Material Number:	562922
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
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The RA3-6B2 monoclonal antibody specifically binds to 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™ BV421 which is part of the BD Horizon Brilliant™ Violet family of dyes. With an Ex Max of 407-nm and Em Max at 421-nm, BD Horizon™ BV421 can be excited by the violet laser and detected in the standard Pacific Blue™ filter set (eg, 450/50-nm filter). BD Horizon™ BV421 conjugates are very bright, often exhibiting a 10 fold improvement in brightness compared to Pacific Blue™ conjugates.

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™ BV421 under optimum conditions, and unconjugated antibody and free BD Horizon™ BV421 were removed.

Application Notes

Application

Flow cytometry	Routinely Tested
Immunofluorescence	Tested During Development

Recommended Assay Procedure:

For optimal and reproducible results, BD Horizon Brilliant Stain Buffer should be used anytime two or more BD Horizon Brilliant dyes are used in the same experiment. Fluorescent dye interactions may cause staining artifacts which may affect data interpretation. The BD Horizon Brilliant Stain Buffer was designed to minimize these interactions. More information can be found in the Technical Data Sheet of the BD Horizon Brilliant Stain Buffer (Cat. No. 563794).

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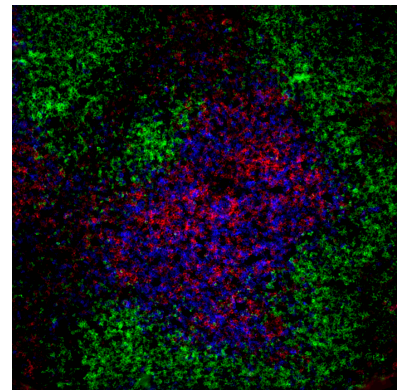
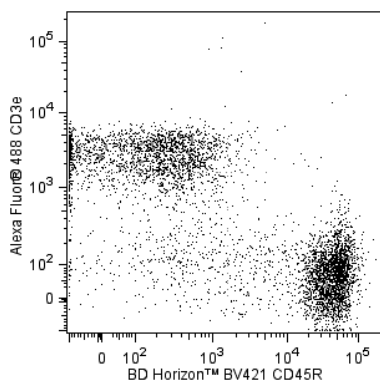
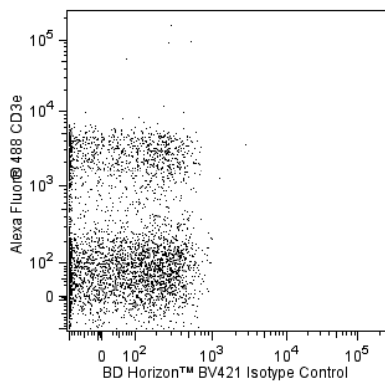
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Multicolor flow cytometric analysis of CD45R/B220 expressed on mouse splenocytes (left and center panels). Splenocytes from a BALB/c mouse were stained with Alexa Fluor® 488 Hamster Anti-Mouse CD3e antibody (Cat. No. 557666) and either BD Horizon™ BV421 Rat IgG2a, κ Isotype Control (Cat. No. 562602; Left Panel) or BD Horizon™ BV421 Rat Anti-Mouse CD45R antibody (Cat. No. 562922; Center Panel). Two-color flow cytometric dot plots show the correlated expression patterns of CD3 versus CD45R (or Ig Isotype control staining) for gated events with the forward and side light-scatter characteristics of viable splenic leucocytes. Flow cytometry was performed using a BD™ LSR II Flow Cytometer System.

Immunofluorescent analysis of CD45R/B220 expression by cells within C57BL/6 mouse spleen (right panel). A mouse spleen cryosection (5 μm) was fixed with BD Cytotfix™ Fixation Buffer (Cat. No. 554655), blocked with 5% goat serum and 1% BSA diluted in 1x PBS, and stained with BD Horizon™ BV421 Rat Anti-Mouse CD45R/B220 antibody (Cat. No. 562922, pseudo-colored green), BD Horizon™ BV480 Rat Anti-Mouse CD4 antibody (Cat. No. 565634, pseudo-colored blue), and Alexa Fluor® 647 Rat-anti-Mouse CD8a (Cat. No. 557682, pseudo-colored red). Images were captured on a standard epifluorescence microscope. Original magnification, 20x.

Suggested Companion Products

Catalog Number	Name	Size	Clone
557666	Alexa Fluor® 488 Hamster Anti-Mouse CD3e	0.1 mg	145-2C11
554656	Stain Buffer (FBS)	500 mL	(none)
562602	BV421 Rat IgG2a, κ Isotype Control	50 μg	R35-95
554657	Stain Buffer (BSA)	500 mL	(none)
564907	DAPI Solution	1 mg	(none)
563794	Brilliant Stain Buffer	100 Tests	(none)
554655	Fixation Buffer	100 mL	(none)
565634	BV480 Rat Anti-Mouse CD4	50 μg	RM4-5
557682	Alexa Fluor® 647 Rat Anti-Mouse CD8a	0.1 mg	53-6.7

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
3. An isotype control should be used at the same concentration as the antibody of interest.
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.
5. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at wwwbdbiosciences.com/colors.
6. Pacific Blue™ is a trademark of Molecular Probes, Inc., Eugene, OR.
7. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
8. BD Horizon Brilliant Violet 421 is covered by one or more of the following US patents: 8,158,444; 8,362,193; 8,575,303; 8,354,239.
9. BD Horizon Brilliant Stain Buffer is covered by one or more of the following US patents: 8,110,673; 8,158,444; 8,575,303; 8,354,239.
10. Please refer to wwwbdbiosciences.com/pharmingen/protocols for technical protocols.

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