

Alexa Fluor[®] 594 anti-mouse/human CD45R/B220 Antibody

Catalog# / Size	103254 / 100 µg
Clone	RA3-6B2
Regulatory Status	RUO
Other Names	B220
Isotype	Rat IgG2a, κ

Description CD45R, also known as B220, is an isoform of CD45. It is a member of the protein tyrosine phosphatase (PTP) family with a molecular weight of approximately 180-240 kD. CD45R is expressed on B cells (at all developmental stages from pro-B cells through mature B cells), activated B cells, and subsets of T and NK cells. CD45R (B220) is also expressed on a subset of abnormal T cells involved in the pathogenesis of systemic autoimmunity in MRL-*Fas*^{lpr} and MRL-*Fas*^{gld} mice. It plays a critical role in TCR and BCR signaling. The primary ligands for CD45 are galectin-1, CD2, CD3, and CD4. CD45R is commonly used as a pan-B cell marker; however, CD19 may be more appropriate for B cell specificity.

Product Details

Verified Reactivity	Mouse, Human
Reported Reactivity	Cat
Antibody Type	Monoclonal
Host Species	Rat
Immunogen	Abelson murine leukemia virus-induced pre-B tumor cells
Formulation	Phosphate-buffered solution, pH 7.2, containing 0.09% sodium azide.
Preparation	The antibody was purified by affinity chromatography and conjugated with Alexa Fluor [®] 594 under optimal conditions.
Concentration	0.5 mg/mL
Storage & Handling	The antibody solution should be stored undiluted between 2°C and 8°C, and protected from prolonged exposure to light. Do not freeze.
Application	IHC-F - Quality tested FC, 3D IHC - Verified
Recommended Usage	Each lot of this antibody is quality control tested by immunofluorescence staining. For immunohistochemistry, a concentration range of 2.5-5 µg/mL is recommended. For immunofluorescence microscopy, a concentration range of 1.25 - 10 µg/mL is recommended. For flow cytometric staining, the suggested use of this reagent is ≤0.25 µg per million cells in 100 µL volume. For 3D immunohistochemistry on formalin-fixed tissues, a concentration of 5.0 µg/mL is suggested. It is recommended that the reagent be titrated for optimal performance for each application. * Alexa Fluor [®] 594 has an excitation maximum of 590 nm, and a maximum emission of 617 nm. Alexa Fluor [®] and Pacific Blue™ are trademarks of Life Technologies Corporation. View full statement regarding label licenses
Application Notes	Clone RA3-6B2 has been described 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. Additional reported applications (for the relevant formats) include: immunoprecipitation ¹ , <i>in vitro</i> and <i>in vivo</i> modulation of B cell responses ^{2,4} , immunohistochemistry of acetone-fixed frozen sections and formalin-fixed paraffin-embedded sections ^{5,6} , and spatial biology (IBEX) ^{14,15} .

Application References

**(PubMed link indicates
BioLegend citation)**

1. Coffman RL. 1982. *Immunol. Rev.* 69:5. (IP)
2. George A, *et al.* 1994. *J. Immunol.* 152:1014. (Activ)
3. Asensi V, *et al.* 1989. *Immunology* 68:204. (Activ)
4. Domiati-Saad R, *et al.* 1993. *J. Immunol.* 151:5936. (Activ)
5. Hata H, *et al.* 2004. *J. Clin. Invest.* 114:582. (IHC)
6. Monteith CE, *et al.* 1996. *Can. J. Vet. Res.* 60:193. (IHC)
7. Shih FF, *et al.* 2006. *J. Immunol.* 176:3438. (FC)
8. Chang C L-T, *et al.* 2007. *J. Immunol.* 178:6984.
9. Fazilleau N, *et al.* 2007. *Nature Immunol.* 8:753.
10. Lang GL, *et al.* 2008. *Blood* 111:2158. [PubMed](#)
11. Charles N, *et al.* 2010. *Nat. Med.* 16:701. (FC) [PubMed](#)
12. del Rio ML, *et al.* 2011. *Transpl. Int.* 24:501. (FC) [PubMed](#)
13. Murakami R, *et al.* 2013. *PLoS One.* 8:73270. [PubMed](#)
14. Radtke AJ, *et al.* 2020. *Proc Natl Acad Sci U S A.* 117:33455-65. (SB) [PubMed](#)
15. Radtke AJ, *et al.* 2022. *Nat Protoc.* 17:378-401. (SB) [PubMed](#)

Product Citations

1. Baptista AP *et al.* 2019. *Immunity.* 50(5):1188-1201 . [PubMed](#)
2. Li S, *et al.* 2020. *Cell Death Dis.* 0.9875. [PubMed](#)
3. Pham THM, *et al.* 2020. *Cell Host & Microbe.* 27(1):54-67.e5.. [PubMed](#)
4. Counoupas C, *et al.* 2020. *NPJ Vaccines.* 0.28125. [PubMed](#)
5. Matryba P, *et al.* 2020. *J Immunol.* 1395:204. [PubMed](#)
6. Wang X, *et al.* 2021. *EMBO J.* 40:e105926. [PubMed](#)
7. Kobia FM, *et al.* 2020. *PLoS Biol.* 18:e3000850. [PubMed](#)
8. Luo L, *et al.* 2021. *J Neuroinflammation.* 18:27. [PubMed](#)
9. Shi H *et al.* 2018. *Immunity.* 49(5):899-914 . [PubMed](#)
10. Goldberg MF *et al.* 2018. *Immunity.* 49(6):1090-1102 . [PubMed](#)
11. Kuhn NF *et al.* 2019. *Cancer cell.* 35(3):473-488 . [PubMed](#)
12. Bidet K, *et al.* 2019. *NPJ Vaccines.* 4:27. [PubMed](#)
13. Song W, *et al.* 2022. *Immunity.* 55:290. [PubMed](#)

RRID

AB_2563229 (BioLegend Cat. No. 103254)

Antigen Details

Structure	Protein tyrosine phosphatase (PTP) family, 180-240 kD
Distribution	B cells, T cell subset, NK cell subset
Function	Phosphatase, T and B cell activation
Ligand/Receptor	Galectin-1, CD2, CD3, CD4
Cell Type	B cells, NK cells, T cells
Biology Area	Cell Biology, Immunology, Inhibitory Molecules, Neuroscience, Neuroscience Cell Markers
Molecular Family	CD Molecules
Antigen References	<ol style="list-style-type: none">1. Barclay A, <i>et al.</i> 1997. <i>The Leukocyte Antigen FactsBook</i> Academic Press.2. Trowbridge IS, <i>et al.</i> 1993. <i>Annu. Rev. Immunol.</i> 12:85.3. Kishihara K, <i>et al.</i> 1993. <i>Cell</i> 74:143.4. Pulido R, <i>et al.</i> 1988. <i>J. Immunol.</i> 140:3851.
Gene ID	19264 5788

Related Protocols

[Immunohistochemistry Protocol for Frozen Sections](#)

[Cell Surface Flow Cytometry Staining Protocol](#)

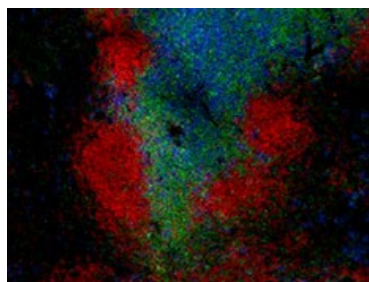
[Immunocytochemistry Staining Protocol](#)

[Ce3D™ Tissue Clearing Kit](#)

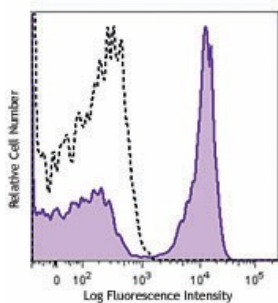
Other Formats

Alexa Fluor® 594 anti-mouse/human CD45R/B220, APC anti-mouse/human CD45R/B220, Biotin anti-mouse/human CD45R/B220, FITC anti-mouse/human CD45R/B220, PE anti-mouse/human CD45R/B220, PE/Cyanine5 anti-mouse/human CD45R/B220, Purified anti-mouse/human CD45R/B220, PE/Cyanine7 anti-mouse/human CD45R/B220, APC/Cyanine7 anti-mouse/human CD45R/B220, Alexa Fluor® 488 anti-mouse/human CD45R/B220, Alexa Fluor® 647 anti-mouse/human CD45R/B220, Pacific Blue™ anti-mouse/human CD45R/B220, Alexa Fluor® 700 anti-mouse/human CD45R/B220, PerCP anti-mouse/human CD45R/B220, PerCP/Cyanine5.5 anti-mouse/human CD45R/B220, Brilliant Violet 421™ anti-mouse/human CD45R/B220, Brilliant Violet 570™ anti-mouse/human CD45R/B220, Brilliant Violet 650™ anti-mouse/human CD45R/B220, Brilliant Violet 605™ anti-mouse/human CD45R/B220, Brilliant Violet 785™ anti-mouse/human CD45R/B220, Brilliant Violet 510™ anti-mouse/human CD45R/B220, Purified anti-mouse/human CD45R/B220 (Maxpar® Ready), Brilliant Violet 711™ anti-mouse/human CD45R/B220, PE/Dazzle™ 594 anti-mouse/human CD45R/B220, APC/Fire™ 750 anti-mouse/human CD45R/B220, Brilliant Violet 750™ anti-mouse/human CD45R/B220, TotalSeq™-A0103 anti-mouse/human CD45R/B220, Spark Blue™ 550 anti-mouse/human CD45R/B220, Spark NIR™ 685 anti-mouse/human CD45R/B220, TotalSeq™-B0103 anti-mouse/human CD45R/B220, Ultra-LEAF™ Purified anti-mouse/human CD45R/B220, TotalSeq™-C0103 anti-mouse/human CD45R/B220, PE/Fire™ 640 anti-mouse/human CD45R/B220, APC/Fire™ 810 anti-mouse/human CD45R/B220, PE/Fire™ 700 anti-mouse/human CD45R/B220, Spark Violet™ 538 anti-mouse/human CD45R/B220, Spark YG™ 581 anti-mouse/human CD45R/B220, Spark YG™ 570 anti-mouse/human CD45R/B220, PE/Fire™ 810 anti-mouse/human CD45R/B220, Spark Blue™ 574 anti-mouse/human CD45R/B220 Antibody, Spark Violet™ 423 anti-mouse/human CD45R/B220 Antibody, Spark Red™ 718 anti-mouse/human CD45R/B220

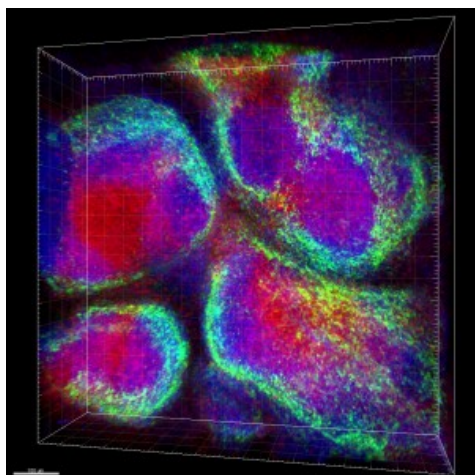
Product Data



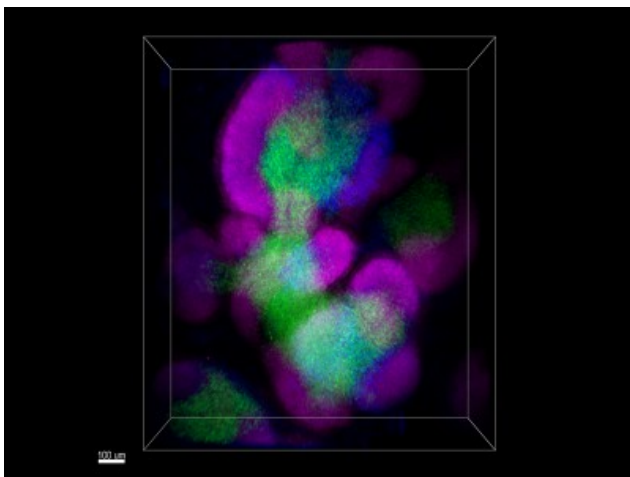
C57BL/6 mouse frozen lymph node section was fixed with 4% paraformaldehyde (PFA) for 10 minutes at room temperature and blocked with 5% FBS plus 5% rat serum for 1 hour at room temperature. Then the section was stained with 2.5 µg/mL of B220 (clone RA3-6B2) Alexa Fluor® 594 (red), 2.5 µg/mL of CD4 (clone RM4-5) Alexa Fluor® 488 (green), and 2.5 µg/mL of CD8 (clone 53-6.7) Alexa Fluor® 647 (blue) overnight at 4°C. The image was captured by 10X objective.



C57BL/6 mouse splenocytes were stained with CD45R/B220 (clone RA3-6B2) Alexa Fluor® 594 (filled histogram) or rat IgG2a, κ Alexa Fluor® 594 isotype control (open histogram). The data was acquired by BD LSRFortessa™ cell analyzer equipped with Yellow-Green Laser (561 nm).



Formalin-fixed, 300 micron-thick mouse spleen section was blocked, permeabilized and stained overnight with CD4 (clone RM4-5) Alexa Fluor® 647 (red), CD169 (Siglec-1)(clone 3D6.112) Alexa Fluor® 488 (green), and CD45R/B220 (clone RA3-6B2) Alexa Fluor® 594 (blue) all at 5 µg/mL, optically cleared, then analyzed at 220 µm imaging depth on a confocal microscope. [Watch the video.](#)



Paraformaldehyde-fixed (4%), 500 μ m-thick mouse spleen section was processed according to the Ce3DTM Tissue Clearing Kit protocol (Cat. No. 427701). The section was costained with anti-mouse CD3 ϵ Antibody (clone 500A2) Alexa Fluor® 488 at 5 μ g/mL (green), and anti-mouse/human CD45R/B220 Antibody (clone RA3-6B2) Alexa Fluor® 594 at 5 μ g/mL (magenta) and counterstained with DAPI (blue). The section was then optically cleared and mounted in a sample chamber. The image was captured with a 10X objective using Zeiss 780 confocal microscope and processed by Imaris image analysis software.

[Watch the video.](#)

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