SONY

Product Data Sheet

Spark Violet™ 538 anti-mouse/human CD45R/B220

Catalog # / Size: 1116420 / 100 µg
1116415 / 25 µg

Clone: RA3-6B2

Isotype: Rat IgG2a, κ

Immunogen: Abelson murine leukemia virus-induced pre-B tumor cells

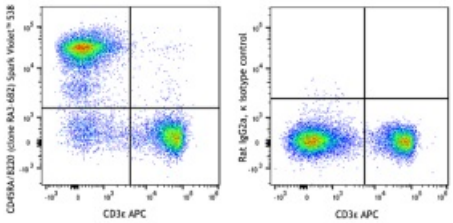
Reactivity: Human, Mouse

Preparation: The antibody was purified by affinity chromatography and conjugated with Spark Violet™ 538 under optimal conditions.

Formulation: Phosphate-buffered solution, pH 7.2, containing 0.09% sodium azide

Workshop Number: 750 under optimal conditions.

Concentration: 0.5



C57BL/6 mouse splenocytes stained with CD3ε APC and CD45R/B220 (clone RA3-6B2) Spark Violet™ 538 (left) or rat IgG2a, k Spark Violet™ 538 isotype control (right).

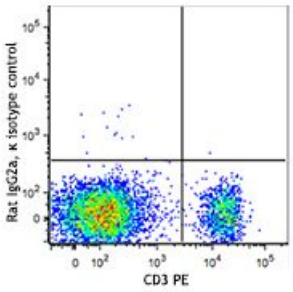
Applications:

Applications: Flow Cytometry

Recommended Usage: Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis. For flow cytometric staining, the suggested use of this reagent is ≤ 1.0 µg per million cells in 100 µL volume. It is recommended that the reagent be titrated for optimal performance for each application.

* Spark Violet™ 538 has a maximum excitation of 396 nm and a maximum emission of 538 nm.

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], *in vitro* and *in vivo* modulation of B cell responses[2-4], and immunohistochemistry of acetone-fixed frozen sections and formalin-fixed paraffin-embedded sections[5,6].



C57BL/6 mouse frozen spleen sections were fixed with 4% paraformaldehyde (PFA) for ten minutes at room temperature and blocked with 5% FBS plus 5% rat/mouse serum for 30 minutes at room temperature. Then the section was stained with 1.25 µg/ml of anti-mouse/human CD45R/B220 (clone RA3-6B2) Brilliant Violet 421™ (blue), anti-mouse CD5 (clone 53-7.3) Alexa Fluor® 594 (red), and anti-mouse F4/80 (clone BM8) Alexa Fluor® 647 (green) overnight at 4°C. The image was captured with a 10X objective.

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Application References:

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

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.

Antigen References:

1. Barclay A, *et al.* 1997. The Leukocyte Antigen FactsBook Academic Press.
2. Trowbridge IS, *et al.* 1993. *Annu. Rev. Immunol.* 12:85.
3. Kishihara K, *et al.* 1993. *Cell* 74:143.
4. Pulido R, *et al.* 1988. *J. Immunol.* 140:3851.

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