Brilliant Violet 421™ anti-mouse CD8a Antibody

Catalog# / Size	100737 / 125 µL 100753 / 50 µg 100738 / 500 µL
Clone	53-6.7
Regulatory Status	RUO
Other Names	T8, Lyt2, Ly-2
Isotype	Rat IgG2a, κ
Description	CD8, also known as Lyt-2, Ly-2, or T8, consists of disulfide-linked α and β chains that form the α(CD8a)/β(CD8b) heterodimer and α/α homodimer. CD8a is a 34 kD protein that belongs to the immunoglobulin family. The CD8 α/β heterodimer is expressed on the surface of most thymocytes and a subset of mature TCR α/β T cells. CD8 expression on mature T cells is non-overlapping with CD4. The CD8 α/α homodimer is expressed on a subset of γ/δ TCR-bearing T cells, NK cells, intestinal intraepithelial lymphocytes, and lymphoid dendritic cells. CD8 is an antigen co-receptor on T cells that interacts with MHC class I on antigen-presenting cells or epithelial cells. CD8 promotes T cell activation through its association with the TCR complex and protein tyrosine kinase lck.

Product Details

Verified Reactivity	Mouse
Antibody Type	Monoclonal
Host Species	Rat
Immunogen	Mouse thymus or spleen
Formulation	Phosphate-buffered solution, pH 7.2, containing 0.09% sodium azide and BSA (origin USA).
Preparation	The antibody was purified by affinity chromatography and conjugated with Brilliant Violet 421™ under optimal conditions.
Concentration	µg sizes: 0.2 mg/mL µL sizes: lot-specific (to obtain lot-specific concentration, please enter the lot number in our Concentration and Expiration Lookup or Certificate of Analysis online tools.)
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	FC - Quality tested IHC - Verified SB - Reported in the literature, not verified in house
Recommended Usage	Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis. For immunofluorescent staining using the µg size, the suggested use of this reagent is ≤0.5 µg per million cells in 100 µl volume. For immunofluorescent staining using µl sizes, the suggested use of this reagent is 5 µl per million cells in 100 µl staining volume or 5 µl per 100 µl of whole blood. It is recommended that the reagent be titrated for optimal performance for each application. Brilliant Violet 421™ excites at 405 nm and emits at 421 nm. The standard bandpass filter 450/50 nm is recommended for detection. Brilliant Violet 421™ is a trademark of Sirigen Group Ltd. Learn more about Brilliant Violet™. This product is subject to proprietary rights of Sirigen Inc. and is made and sold under license from Sirigen Inc. The purchase of this product conveys to the buyer a non-transferable right to use the purchased product for research purposes only. This product may not be resold or incorporated in any manner into another product for resale. Any use for therapeutics or diagnostics is strictly prohibited. This product is covered by U.S. Patent(s), pending patent applications and foreign equivalents.

Excitation Laser

Violet Laser (405 nm)

Application Notes

Clone 53-6.7 antibody competes with clone 5H10-1 antibody for binding to thymocytes[3]. The 53-6.7 antibody has been reported to block antigen presentation via MHC class I and inhibit T cell responses to IL-2. This antibody has also been used for depletion of CD8a$^+$ cells. Additional reported applications (for the relevant formats) include: immunoprecipitation[1,3], *in vivo* and *in vitro* cell depletion[2,10,15], inhibition of CD8 T cell proliferation[3], blocking of cytotoxicity[3,4], immunohistochemical staining[5,6] of acetone-fixed frozen sections and zinc-fixed paraffin-embedded sections, and spatial biology (IBEX)[29,30]. Clone 53-6.7 is not recommended for immunohistochemistry of formalin-fixed paraffin sections. The Ultra-LEAF™ purified antibody (Endotoxin < 0.01 EU/µg, Azide-Free, 0.2 µm filtered) is recommended for functional assays or in vivo studies (Cat No. 100746).

Additional Product Notes

Iterative Bleaching Extended multi-pleXity (IBEX) is a fluorescent imaging technique capable of highly-multiplexed spatial analysis. The method relies on cyclical bleaching of panels of fluorescent antibodies in order to image and analyze many markers over multiple cycles of staining, imaging, and, bleaching. It is a community-developed open-access method developed by the Center for Advanced Tissue Imaging (CAT-I) in the National Institute of Allergy and Infectious Diseases (NIAID, NIH).

Application References

(PubMed link indicates BioLegend citation)

1. Ledbetter JA, *et al.* 1979. *Immunol. Rev.* 47:63. (IHC, IP)
2. Hathcock KS. 1991. *Current Protocols in Immunology.* 3.4.1. (Deplete)
3. Takahashi K, *et al.* 1992. *P. Natl. Acad. Sci. USA* 89:5557. (Block, IP)
4. Ledbetter JA, *et al.* 1981. *J. Exp. Med.* 153:1503. (Block)
5. Hata H, *et al.* 2004. *J. Clin. Invest.* 114:582. (IHC)
6. Fan WY, *et al.* 2001. *Exp. Biol. Med.* 226:1045. (IHC)
7. Shih FF, *et al.* 2006. *J. Immunol.* 176:3438. (FC)
8. Kamimura D, *et al.* 2006. *J. Immunol.* 177:306.
9. Bouwer HGA, *et al.* 2006. *P. Natl. Acad. Sci. USA* 103:5102. (FC, Deplete)
10. Kao C, *et al.* 2005. *Int. Immunol.* 17:1607. PubMed
11. Ko SY, *et al.* 2005. *J. Immunol.* 175:3309. (FC) PubMed
12. Rasmussen JW, *et al.* 2006. *Infect. Immun.* 74:6590. PubMed
13. Lee CH, *et al.* 2009. *Clin. Cancer Res.* PubMed
14. Geiben-Lynn R, *et al.* 2008. *Blood* 112:4585. (Deplete) PubMed
15. Kingeter LM, *et al.* 2008. *J. Immunol.* 181:6244. PubMed
16. Guo Y, *et al.* 2008. *Blood* 112:480. PubMed
17. Andrews DM, *et al.* 2008. *J. Virol.* 82:4931. PubMed
18. Britschqui MR, *et al.* 2008. *J. Immunol.* 181:7681. PubMed
19. Kenna TJ, *et al.* 2008. *Blood* 111:2091. PubMed
20. Jordan JM, *et al.* 2008. *Infect. Immun.* 76:3717. PubMed
21. Todd DJ, *et al.* 2009. *J. Exp. Med.* 206:2151. PubMed
22. Bankoti J, *et al.* 2010. *Toxicol. Sci.* 115:422. (FC) PubMed
23. Medyouf H, *et al.* 2010. *Blood* 115:1175. PubMed
24. Riedl P, *et al.* 2009. *J. Immunol.* 183:370. PubMed
25. Apte SH, *et al.* 2010. *J. Immunol.* 185:998. PubMed
26. Bankoti J, *et al.* 2010. *Toxicol. Sci.* 115:422. (FC) PubMed
27. del Rio ML, *et al.* 2011. *Transpl. Int.* 24:501. (FC) PubMed
28. Cui L, *et al.* 2015. *J Control Release.* 206:220. PubMed
29. Radtke AJ, *et al.* 2020. *Proc Natl Acad Sci U S A.* 117:33455-65. (SB) PubMed
30. Radtke AJ, *et al.* 2022. *Nat Protoc.* 17:378-401. (SB) PubMed

Product Citations

1. Pavelko K, *et al.* 2017. Front Immunol. . 10.3389/fimmu.2017.01532. PubMed
2. Klepsch V, *et al.* 2018. Nat Commun. 9:1538. PubMed
3. Zwick M, *et al.* 2019. Front Immunol. 10:222. PubMed
4. Groza D, *et al.* 2018. Oncoimmunology. 7:e1424676. PubMed
5. LaFleur MW, *et al.* 2019. Nat Commun. 10:1668. PubMed
6. Franks SE, *et al.* 2019. J Immunol. 202:3381. PubMed
7. Baptista AP *et al.* 2019. Immunity. 50(5):1188-1201 . PubMed
8. Jayachandran R, *et al.* 2019. Immunity. 50:152. PubMed
9. Young A, *et al.* 2017. Cancer Res. 77:4684. PubMed
10. Parks CA, *et al.* 2019. Proc Natl Acad Sci U S A. 116:3136. PubMed
11. Kacherovsky N, *et al.* 2019. Nat Biomed Eng. 0.66875. PubMed
12. Horkova V, *et al.* 2020. Cell Reports. 30(5):1504-1514.e7.. PubMed
13. LaFleur MW, *et al.* 2019. Nat Immunol. 20:1335. PubMed
14. Stokes J, *et al.* 2020. Oncoimmunology. 9:1758011. PubMed
15. Briukhovetska D, *et al.* 2020. Frontiers in Immunology. 1.428472222. PubMed
16. Fukushima T, *et al.* 2019. Cell Rep. 29:4144. PubMed
17. Siamishi I, *et al.* 2020. Cell Reports. 31(11):107756. PubMed
18. Stathopoulou C, *et al.* 2020. Immunity. 49(2):247-263.e7.. PubMed
19. Hoyer FF, *et al.* 2020. Immunity. 51(5):899-914.e7.. PubMed
20. Lees JG, *et al.* 2020. PLoS One. 15:e0238164. PubMed
21. Sinclair LV, *et al.* 2020. Immunometabolism. 2:e200029. PubMed
22. Fulham MA, *et al.* 2019. Am J Physiol Cell Physiol. 317:C687. PubMed
23. Chen Z, *et al.* 2014. Cancer Immunol Res. 2:911. PubMed
24. Ballet R, *et al.* 2014. PLoS Pathog. 10:1004550. PubMed
25. Hu-Lieskovan S, *et al.* 2015. Sci Transl Med. 7:279. PubMed
26. Ding Z, Dahlin J 2016. Sci Rep. 6:28290. PubMed

27. LM S, *et al.* 2016. Cell Rep. 16(12): 3286-96. PubMed
28. Corrado M, *et al.* 2020. Cell Metab. 32:981. PubMed
29. Köchl R, *et al.* 2020. Elife. 9:00. PubMed
30. Iwanami N, *et al.* 2020. iScience. 23:101260. PubMed
31. Evgin L, *et al.* 2020. Nat Commun. 2.671527778. PubMed
32. Fan Z, *et al.* 2020. EMBO Mol Med. 12:e11571. PubMed
33. Wei Z, *et al.* 2021. Nat Commun. 0.805555556. PubMed
34. Lutes LK, *et al.* 2021. eLife. 10:00. PubMed
35. Acharya N, *et al.* 2020. Immunity. 53(3):658-671.e6. PubMed
36. Dammeijer F, *et al.* 2020. Cancer Cell. 38(5):685-700.e8. PubMed
37. Ringel AE, *et al.* 2020. Cell. 183(7):1848-1866.e26. PubMed
38. Mogilenko DA, *et al.* 2020. Immunity. 54(1):99-115.e12. PubMed
39. Bambouskova M, *et al.* 2021. Cell Reports. 34(10):108756. PubMed
40. Delacher M, *et al.* 2021. Immunity. 54(4):702-720.e17. PubMed
41. Levine LS, *et al.* 2021. Immunity. 54(4):829-844.e5. PubMed
42. Mitchell JE, *et al.* 2021. Cell Reports. 35(2):108966. PubMed
43. Giampazolias E, *et al.* 2021. Cell. . PubMed

RRID

AB_10897101 (BioLegend Cat. No. 100737)
AB_2562558 (BioLegend Cat. No. 100753)
AB_11204079 (BioLegend Cat. No. 100738)

Antigen Details

Structure

Ig superfamily, CD8α chain, 34 kD

Distribution

Most thymocytes, T cell subset, some NK cells, lymphoid dendritic cells

Function

Co-receptor for TCR

Ligand/Receptor

MHC class I molecule

Antigen References

1. Barclay A, *et al.* 1997. The Leukocyte Antigen FactsBook Academic Press.
2. Zamoyska R. 1994. *Immunity* 1:243.
3. Ellmeier W, *et al.* 1999. *Annu. Rev. Immunol.* 17:523.

Gene ID

12525

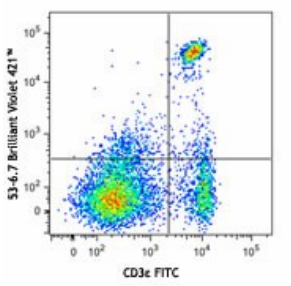
Related Protocols

Cell Surface Flow Cytometry Staining Protocol

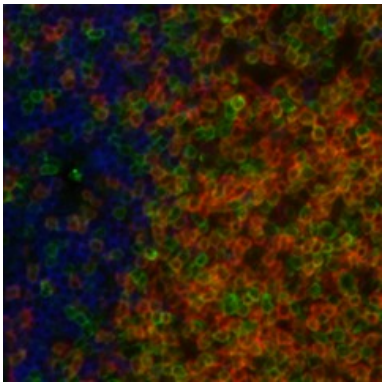
Other Formats

APC anti-mouse CD8a, Biotin anti-mouse CD8a, FITC anti-mouse CD8a, PE anti-mouse CD8a, PE/Cyanine5 anti-mouse CD8a, Purified anti-mouse CD8a, PE/Cyanine7 anti-mouse CD8a, APC/Cyanine7 anti-mouse CD8a, Alexa Fluor® 488 anti-mouse CD8a, Alexa Fluor® 647 anti-mouse CD8a, Pacific Blue™ anti-mouse CD8a, Alexa Fluor® 700 anti-mouse CD8a, PerCP/Cyanine5.5 anti-mouse CD8a, PerCP anti-mouse CD8a, Brilliant Violet 421™ anti-mouse CD8a, Brilliant Violet 570™ anti-mouse CD8a, Brilliant Violet 650™ anti-mouse CD8a, Brilliant Violet 605™ anti-mouse CD8a, Ultra-LEAF™ Purified anti-mouse CD8a, Brilliant Violet 711™ anti-mouse CD8a, Brilliant Violet 785™ anti-mouse CD8a, Brilliant Violet 510™ anti-mouse CD8a, Purified anti-mouse CD8a (Maxpar® Ready), Alexa Fluor® 594 anti-mouse CD8a, PE/Dazzle™ 594 anti-mouse CD8a, APC/Fire™ 750 anti-mouse CD8a, GolnVivo™ Purified anti-mouse CD8a, TotalSeq™-A0002 anti-mouse CD8a, Spark Blue™ 550 anti-mouse CD8a, Spark NIR™ 685 anti-mouse CD8a, TotalSeq™-C0002 anti-mouse CD8a, TotalSeq™-B0002 anti-mouse CD8a, Spark YG™ 570 anti-mouse CD8a, PE/Fire™ 640 anti-mouse CD8a, PE/Fire™ 700 anti-mouse CD8a, Spark Blue™ 574 anti-mouse CD8a Antibody, Spark Violet™ 423 anti-mouse CD8a Antibody, Spark UV™ 387 anti-mouse CD8a

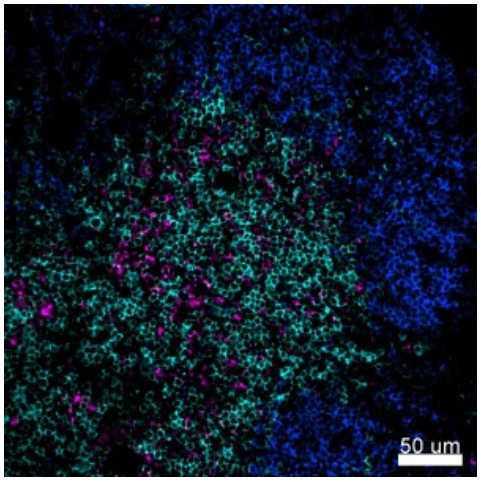
Product Data



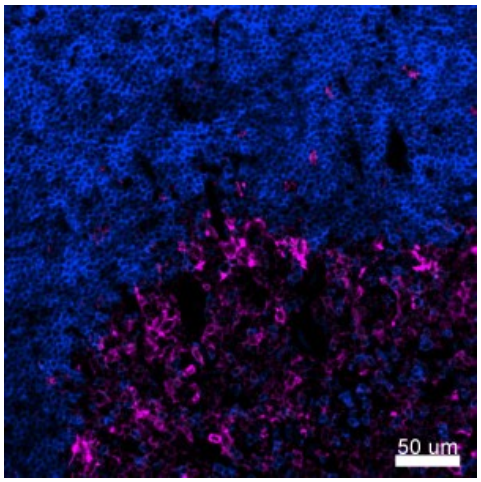
C57BL/6 mouse splenocytes were stained with CD3ε FITC and CD8a (clone 53-6.7) Brilliant Violet 421™. Quadrant gating was based on the rat IgG2a, κ Brilliant Violet 421™ isotype control.



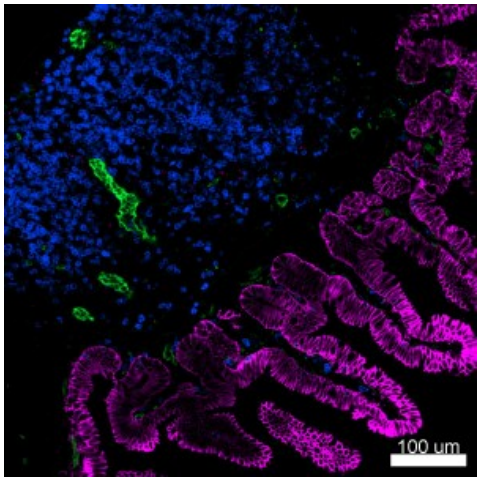
BL6 mouse lymph nodes, fixed O/N in PLP, blocked with 10% rat serum, stained with CD8a-BV421 (red), B220-Alexa Fluor® 647 (blue), and TCRβ-Alexa Fluor® 488 (green) in 1% BSA and 0.1% Tween-20 in PBS. Images were acquired with an automated widefield microscope (Nikon Eclipse Ti) and a CCD camera (QImaging Retiga 2000R). Emitted light was collected through 440/40, 525/50, and 700/75 nm bandpass filters. Images provided by Ann Haberman and Christine Podolski, Yale University.



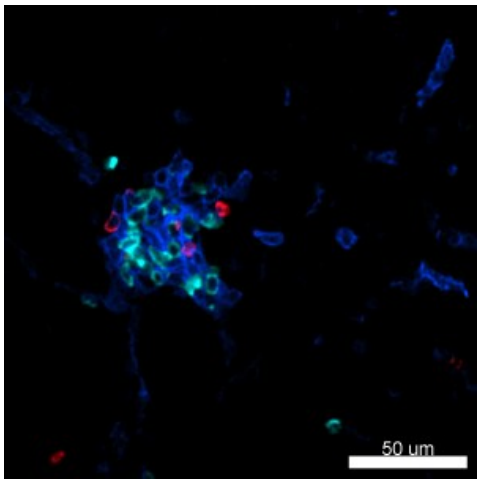
Confocal image of C57BL/6 mouse spleen sample acquired using the IBEX method of highly multiplexed antibody-based imaging: CD4 (cyan), CD8 (magenta), and IgD (blue) in Cycle 1. Tissues were prepared using ~1% (vol/vol) formaldehyde and a detergent. Following fixation, samples are immersed in 30% (wt/vol) sucrose for cryoprotection. Images are courtesy of Drs. Andrea J. Radtke and Ronald N. Germain of the Center for Advanced Tissue Imaging (CAT-I) in the National Institute of Allergy and Infectious Diseases (NIAID, NIH).



Confocal image of C57BL/6 mouse thymus sample acquired using the IBEX method of highly multiplexed antibody-based imaging: CD8 (blue) in Cycle 2 and CD11c (magenta) in Cycle 3. Tissues were prepared using ~1% (vol/vol) formaldehyde and a detergent. Following fixation, samples are immersed in 30% (wt/vol) sucrose for cryoprotection. Images are courtesy of Drs. Andrea J. Radtke and Ronald N. Germain of the Center for Advanced Tissue Imaging (CAT-I) in the National Institute of Allergy and Infectious Diseases (NIAID, NIH).



Confocal image of C57BL/6 mouse small intestine sample acquired using the IBEX method of highly multiplexed antibody-based imaging: EpCAM (magenta) in Cycle 1, CD8 (blue) in Cycle 1, and CD31 (green) in Cycle 2. Tissues were prepared using ~1% (vol/vol) formaldehyde and a detergent. Following fixation, samples are immersed in 30% (wt/vol) sucrose for cryoprotection. Images are courtesy of Drs. Andrea J. Radtke and Ronald N. Germain of the Center for Advanced Tissue Imaging (CAT-I) in the National Institute of Allergy and Infectious Diseases (NIAID, NIH).



Confocal image of C57BL/6 mouse liver sample acquired using the IBEX method of highly multiplexed antibody-based imaging: CD8 (cyan) in Cycle 2, CD44 (blue) in Cycle 2, and NK1.1 (red) in Cycle 3. Tissues were prepared using ~1% (vol/vol) formaldehyde and a detergent. Following fixation, samples are immersed in 30% (wt/vol) sucrose for cryoprotection. Images are courtesy of Drs. Andrea J. Radtke and Ronald N. Germain of the Center for Advanced Tissue Imaging (CAT-I) in the National Institute of Allergy and Infectious Diseases (NIAID, NIH).

BioLegend Inc., 8999 BioLegend Way, San Diego, CA 92121 www.biolegend.com
Toll-Free Phone: 1-877-Bio-Legend (246-5343) Phone: (858) 768-5800 Fax: (877) 455-9587