# Alexa Fluor® 647 anti-mouse CD8a Antibody

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| **Catalog# / Size** | 100727 / 25 µg<br>100724 / 100 µg |
| **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

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| **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. |
| **Preparation** | The antibody was purified by affinity chromatography and conjugated with Alexa Fluor® 647 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** | FC - Quality tested<br>IHC-F, 3D IHC - Verified<br>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 flow cytometric staining, the suggested use of this reagent is ≤ 0.25 µg per million cells in 100 µL volume. For immunohistochemistry on frozen tissue sections, a concentration range of 2.5 - 5.0 µg/mL is suggested. 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.<br><br>* Alexa Fluor® 647 has a maximum emission of 668 nm when it is excited at 633nm / 635nm.<br><br>Alexa Fluor® and Pacific Blue™ are trademarks of Life Technologies Corporation.<br><br>View full statement regarding label licenses |
| **Excitation Laser** | Red Laser (633 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**


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**Product Citations**


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**RRID** AB_493424 (BioLegend Cat. No. 100727)
AB_389326 (BioLegend Cat. No. 100724)

## 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**

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**Gene ID** 12525

## Related Protocols

Immunohistochemistry Protocol for Frozen Sections

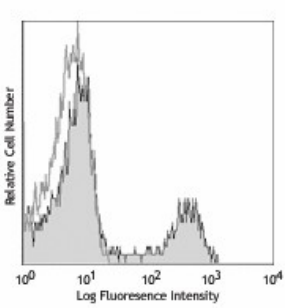
Cell Surface Flow Cytometry Staining Protocol

Ce3D™ Tissue Clearing Kit

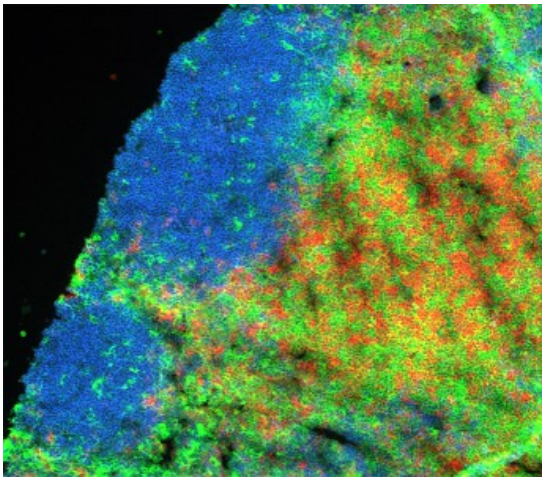
## Other Formats

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, GoInVivo™ 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

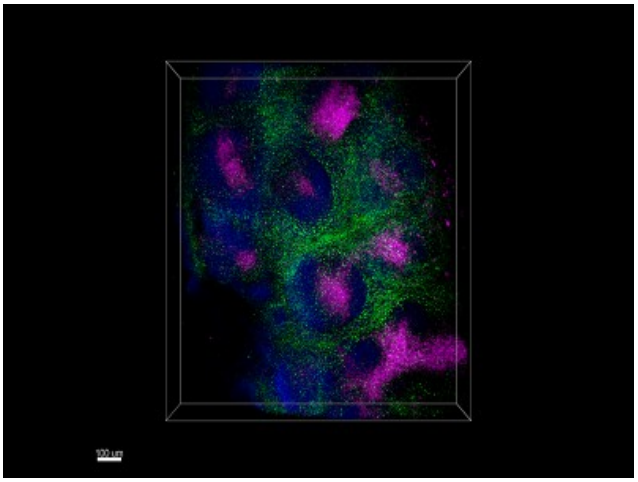
## Product Data



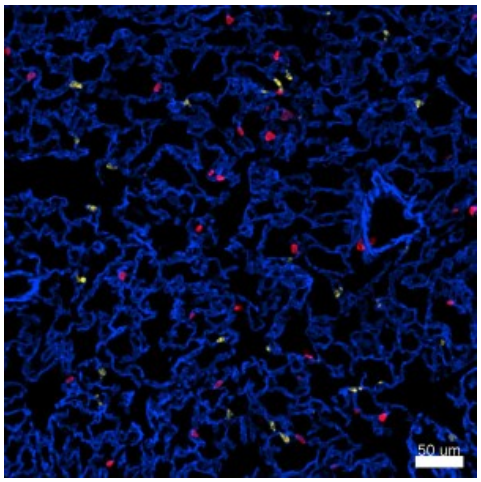

C57BL/6 mouse splenocytes were stained with CD8 (clone 53-6.7) Alexa Fluor® 647 (filled histogram) or rat IgG2a, κ Alexa Fluor® 647 isotype control (open histogram).



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 5 µg/ml of B220 (clone RA3-6B2) Alexa Fluor® 594 (blue), 5 µg/ml of CD8 (clone 53-6.7) Alexa Fluor® 647 (red), and 5 µg/ml of CD4 (clone GK1.5) Alexa Fluor® 488 (green) overnight at 4ËšC. The image was captured by 10X objective.



Paraformaldehyde-fixed (1%), 500 µm-thick mouse spleen section was processed according to the Ce3DTM Tissue Clearing Kit protocol (cat. no. 427701). The section was costained with anti-mouse CD68 Antibody (clone FA-11) Alexa Fluor® 488 at 5 µg/mL (green), and anti-mouse CD8a Antibody (clone 53-6.7) Alexa Fluor® 647 at 5 µg/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.




Confocal image of C57BL/6 mouse lung sample acquired using the IBEX method of highly multiplexed antibody-based imaging: Ly-6G (yellow) in Cycle 2, CD31 (blue) in Cycle 3, and CD8 (red) in Cycle 4. 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).

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