Alexa Fluor® 594 anti-mouse CD3 Antibody

Catalog# / Size	100240 / 100 µg
Clone	17A2
Regulatory Status	RUO
Other Names	T cell antigen receptor complex, T3
Isotype	Rat IgG2b, κ
Description	CD3, also known as T3, is a member of the Ig superfamily and primarily expressed on T cells, NK-T cells, and at different levels on thymocytes during T cell differentiation. CD3 is composed of CD3ε, δ, γ and ζ chains. It forms a TCR complex by associating with TCR α/β or γ/δ chains. CD3 plays a critical role in TCR signal transduction, T cell activation, and antigen recognition by binding the peptide/MHC antigen complex

Product Details

Verified Reactivity	Mouse
Antibody Type	Monoclonal
Host Species	Rat
Immunogen	γδTCR-positive T-T hybridoma D1
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 SB - Reported in the literature, not verified in house
Recommended Usage	Each lot of this antibody is quality control tested by immunohistochemical staining on frozen tissue sections. For immunohistochemistry, a concentration range of 1.0 - 5.0 µg/mL is suggested. 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	Additional reported application (for relevant formats) include: spatial biology (IBEX)[1,2].
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. Radtke AJ, *et al.* 2020. *Proc Natl Acad Sci U S A.* 117:33455-65. (SB) PubMed 2. Radtke AJ, *et al.* 2022. *Nat Protoc.* 17:378-401. (SB) PubMed

Product Citations

1. Soon MSF, *et al.* 2020. Nat Immunol. 1.984027778. PubMed
2. Matsumoto R, *et al.* 2021. Front Pharmacol. 12:715752. PubMed
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4. Waide ML, *et al.* 2020. Cell Rep. 33:108503. PubMed
5. Menzel L, *et al.* 2021. Cell Rep. 37:109878. PubMed
6. Liu M, *et al.* 2020. Nature. 587:115. PubMed
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8. Prados A, *et al.* 2021. Nat Immunol. 22:510. PubMed
9. Wong HS, *et al.* 2021. Cell. . PubMed
10. Wennerberg E, *et al.* 2022. Sci Transl Med. 14:eabe8195. PubMed
11. Chung YM, *et al.* 2021. J Immunother Cancer. 9:. PubMed
12. Qi S *et al.* 2016. eLife. 5 pii: e14756. PubMed
13. Palacio L, *et al.* 2019. Aging Cell. 18(4):e12971. PubMed
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15. Wang X, *et al.* 2021. EMBO J. 40:e105926. PubMed
16. Pantelidou C, *et al.* 2022. NPJ Breast Cancer. 8:102. PubMed
17. Lederer K, *et al.* 2020. Immunity. 53(6):1281-1295.e5. PubMed
18. Lal JC, *et al.* 2021. Breast Cancer Res. 23:83. PubMed

RRID AB_2563427 (BioLegend Cat. No. 100240)

Antigen Details

Structure Ig superfamily, CD3/TCR, 20 kD

Distribution Thymocytes (differentiation dependent), mature T cells, NK-T cells

Function Antigen recognition, TCR signal transduction, T cell activation

Ligand/Receptor Peptide antigen/MHC-complex

Antigen References

1. Barclay A, *et al.* 1997. The Leukocyte Antigen FactsBook Academic Press.
2. Davis MM. 1990. *Annu. Rev. Biochem.* 59:475.
3. Weiss A, *et al.* 1994. *Cell* 76:263.

Gene ID 12502

Related Protocols

Immunohistochemistry Protocol for Frozen Sections

Cell Surface Flow Cytometry Staining Protocol

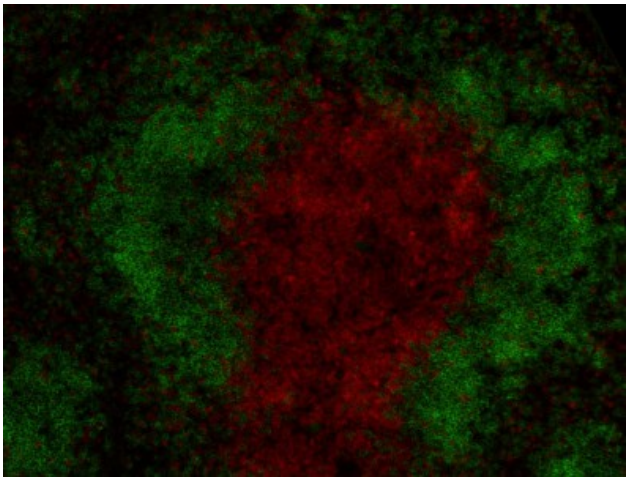
Immunocytochemistry Staining Protocol

Ce3D™ Tissue Clearing Kit

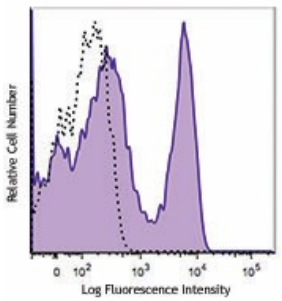
Other Formats

FITC anti-mouse CD3, PE anti-mouse CD3, Purified anti-mouse CD3, Alexa Fluor® 647 anti-mouse CD3, Alexa Fluor® 488 anti-mouse CD3, Pacific Blue™ anti-mouse CD3, Alexa Fluor® 700 anti-mouse CD3, PerCP/Cyanine5.5 anti-mouse CD3, PE/Cyanine7 anti-mouse CD3, APC/Cyanine7 anti-mouse CD3, Brilliant Violet 421™ anti-mouse CD3, Brilliant Violet 570™ anti-mouse CD3, Brilliant Violet 650™ anti-mouse CD3, Brilliant Violet 785™ anti-mouse CD3, Brilliant Violet 510™ anti-mouse CD3, APC anti-mouse CD3, Ultra-LEAF™ Purified anti-mouse CD3, Brilliant Violet 605™ anti-mouse CD3, Alexa Fluor® 594 anti-mouse CD3, Brilliant Violet 711™ anti-mouse CD3, Biotin anti-mouse CD3, PE/Dazzle™ 594 anti-mouse CD3, APC/Fire™ 750 anti-mouse CD3, Brilliant Violet 750™ anti-mouse CD3, TotalSeq™-A0182 anti-mouse CD3, TotalSeq™-B0182 anti-mouse CD3, Spark Blue™ 550 anti-mouse CD3, Spark NIR™ 685 anti-mouse CD3, TotalSeq™-C0182 anti-mouse CD3, APC/Fire™ 810 anti-mouse CD3, PE/Fire™ 640 anti-mouse CD3, Spark YG™ 570 anti-mouse CD3, PE/Fire™ 700 anti-mouse CD3, PE/Cyanine5 anti-mouse CD3, Spark Blue™ 574 anti-mouse CD3 Antibody, Spark Violet™ 423 anti-mouse CD3, PE/Fire™ 810 anti-mouse CD3, Spark Red™ 718 anti-mouse CD3

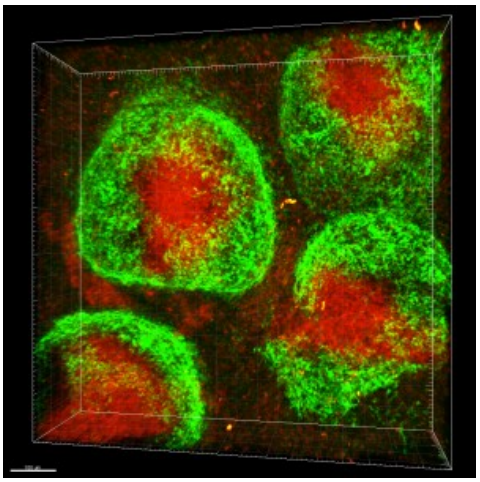
Product Data



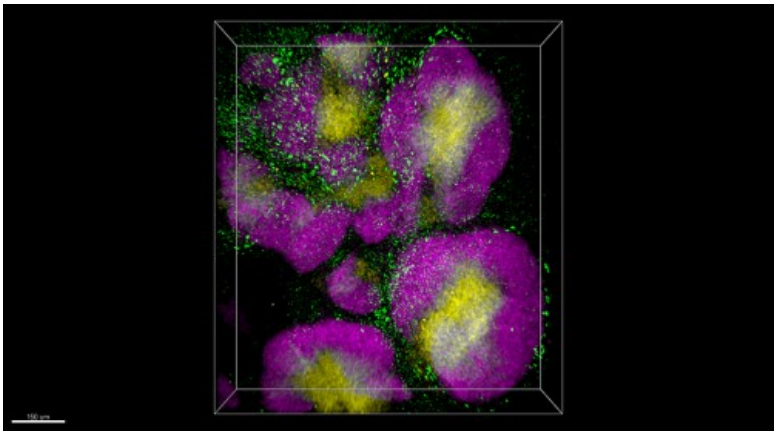
C57BL/6 mouse frozen spleen section was fixed with 4% paraformaldehyde (PFA) for 10 minutes at room temperature and blocked with 5% FBS plus 5% rat serum for 1 hour at room temperature. Then the section was stained with 2.5 µg/mL of CD3 (clone 17A2) Alexa Fluor® 594 (red) and 2.5 µg/mL of B220 (clone RA3-6B2) Alexa Fluor® 488 (green) overnight at 4°C. The image was captured by 10X objective.



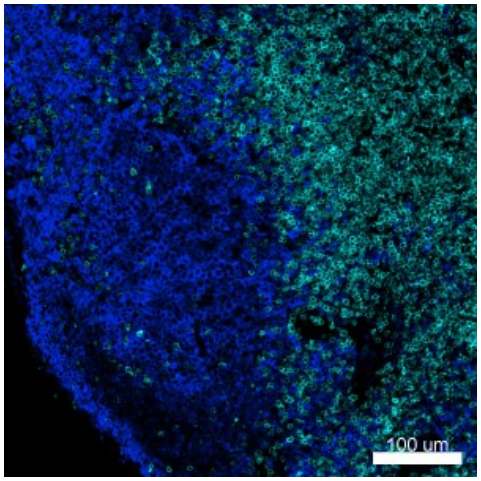
C57BL/6 mouse splenocytes were stained with CD3 (clone 17A2) Alexa Fluor® 594 (filled histogram). The data was acquired by BD LSRFortessa™ cell analyzer equipped with Yellow-Green Laser (561 nm).



Formalin-fixed, 300 micron-thick mouse spleen section was blocked, permeabilized and stained overnight with CD3 (clone 17A2) Alexa Fluor® 594 (red), CD169 (Siglec-1)(clone 3D6.112) Alexa Fluor® 647 (green) both at 5 µg/mL, optically cleared, then analyzed at 225 µm imaging depth on a confocal microscope. Watch the video.



Paraformaldehyde-fixed (4%), 500 µm-thick mouse spleen section was processed according to the Ce3DTM Tissue Clearing Kit protocol (cat. no. 47701). The section was costained with anti-mouse CD68 Antibody (clone FA-11) Alexa Fluor® 488 at 5 µg/mL (green), anti-mouse CD3 Antibody (clone 17A2) Alexa Fluor® 594 at 5 µg/mL (yellow), and anti-mouse IgD Antibody (clone 11-26c.2a) Alexa Fluor® 647 at 5 µg/mL (magenta). 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.



Mice were injected subcutaneously with sheep red blood cells in a volume of 25 □l per site on days 0 and 4 and harvested on day 11. Confocal image of C57BL/6 mouse lymph node acquired using the IBEX method of highly multiplexed antibody-based imaging: CD3 (cyan) in Cycle 1 and MHCII (blue) in Cycle 10. 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