Alexa Fluor® 594 anti-human CD3 Antibody

Catalog# / Size	300446 / 100 µg
Clone	UCHT1
Regulatory Status	RUO
Workshop	III 471
Other Names	T3, CD3ε
Isotype	Mouse IgG1, κ
Description	CD3ε is a 20 kD chain of the CD3/T-cell receptor (TCR) complex which is composed of two CD3ε, one CD3γ, one CD3δ, one CD3ζ (CD247), and a T-cell receptor (α/β or γ/δ) heterodimer. It is found on all mature T cells, NKT cells, and some thymocytes. CD3, also known as T3, is a member of the immunoglobulin superfamily that plays a role in antigen recognition, signal transduction, and T cell activation.

Product Details

Verified Reactivity	Human
Reported Reactivity	Chimpanzee
Antibody Type	Monoclonal
Host Species	Mouse
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	ICC - Quality tested FC - Verified SB - Reported in the literature, not verified in house
Recommended Usage	Each lot of this antibody is quality control tested by immunocytochemistry. For immunocytochemistry, a concentration range of 2.5 - 10 µg/mL is recommended. 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
Excitation Laser	Green Laser (532 nm)/Yellow-Green Laser (561 nm)
Application Notes	Additional reported applications (for the relevant formats) include: immunohistochemical staining of acetone-fixed frozen sections[4,6,7] and formalin-fixed paraffin-embedded sections[11], immunoprecipitation[1], activation of T cells[2,3,5], Western blotting[9], and spatial biology (IBEX)[16,17]. The LEAF™ purified antibody (Endotoxin < 0.1 EU/µg, Azide-Free, 0.2 µm filtered) is recommended for functional assays (Cat. No. 300413, 300414, and 300432). For highly sensitive assays, we recommend Ultra-LEAF™ purified antibody (Cat. No. 300437, 300438, 300465, 300466, 300473, 300474) with a lower endotoxin limit than standard LEAF™ purified antibodies (Endotoxin < 0.01 EU/µg).
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).

View more applications data for this product in our Scientific Poster Library.

Application References

1. Salmeron A, *et al.* 1991. *J. Immunol.* 147:3047. (IP)
2. Graves J, *et al.* 1991. *J. Immunol.* 146:2102. (Activ)
3. Lafont V, *et al.* 2000. *J. Biol. Chem.* 275:19282. (Activ)
4. Ryschich E, *et al.* 2003. *Tissue Antigens* 62:48. (IHC)
5. Thompson AG, *et al.* 2004. *J. Immunol.* 173:1671. (Activ)
6. Sakkas LI, *et al.* 1998. *Clin. Diagn. Lab. Immun.* 5:430. (IHC)
7. Mack CL, *et al.* 2004. *Pediatr. Res.* 56:79. (IHC)
8. Thakral D, *et al.* 2008. *J. Immunol.* 180:7431. (FC) PubMed
9. Van Dongen JJM, *et al.* 1988. *Blood* 71:603. (WB)
10. Yoshino N, *et al.* 2000. *Exp. Anim. (Tokyo)* 49:97. (FC)
11. Pollard, K. *et al.* 1987. *J. Histochem. Cytochem.* 35:1329. (IHC)
12. Luckashenak N, *et al.* 2013. *J. Immunol.* 190:27. PubMed
13. Laurent AJ, *et al.* 2014. *PLoS One.* 9:103683. PubMed
14. Li J, *et al.* 2015. *Cancer Res.* 75:508. PubMed
15. Stoeckius M, *et al.* 2017. *Nat. Methods.* 14:865-868. (PG)
16. Radtke AJ, *et al.* 2020. *Proc Natl Acad Sci USA.* 117:33455-33465. (SB) PubMed
17. Radtke AJ, *et al.* 2022. *Nat Protoc.* 17:378-401. (SB) PubMed

Product Citations

1. Emmons TR, *et al.* 2021. Cancer Immunol Res. 9:790. PubMed
2. Mehta AK, *et al.* 2021. Nat Cancer. 2:66. PubMed
3. Su Y, *et al.* 2021. Cancers (Basel). 13:. PubMed

RRID

AB_2563236 (BioLegend Cat. No. 300446)

Antigen Details

Structure

Ig superfamily, with the subunits of CD3γ, CD3δ, CD3ζ (CD247) and TCR (α/β or γ/δ) forms CD3/TCR complex, 20 kD

Distribution

Mature T and NK T cells, thymocyte differentiation

Function

Antigen recognition, signal transduction, T cell activation

Ligand/Receptor

Peptide antigen bound to MHC

Cell Type

NKT cells, T cells, Thymocytes, Tregs

Biology Area

Immunology, Innate Immunity

Molecular Family

CD Molecules, TCRs

Antigen References

1. Barclay N, *et al.* 1993. The Leucocyte FactsBook. Academic Press. San Diego.
2. Beverly P, *et al.* 1981. *Eur. J. Immunol.* 11:329.
3. Lanier L, *et al.* 1986. *J. Immunol.* 137:2501-2507.

Gene ID

916

Related Protocols

Cell Surface Flow Cytometry Staining Protocol

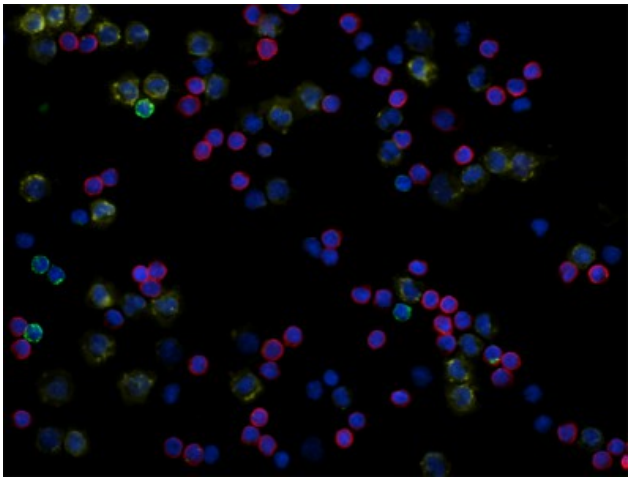
Immunocytochemistry Staining Protocol

Other Formats

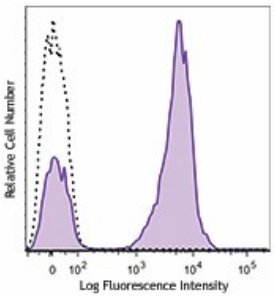
APC anti-human CD3, Biotin anti-human CD3, FITC anti-human CD3, PE anti-human CD3, PE/Cyanine5 anti-human CD3, Purified anti-human CD3, Alexa Fluor® 647 anti-human CD3, Alexa Fluor® 488 anti-human CD3, Pacific Blue™ anti-human CD3, PE/Cyanine7 anti-human CD3, Alexa Fluor® 700 anti-human CD3, APC/Cyanine7 anti-human CD3, PerCP anti-human CD3, PerCP/Cyanine5.5 anti-human CD3, Brilliant Violet 421™ anti-human CD3, Brilliant Violet 570™ anti-human CD3, Ultra-LEAF™ Purified anti-human CD3, Purified anti-human CD3 (Maxpar® Ready), Alexa Fluor® 594 anti-human CD3, PE/Dazzle™ 594 anti-

human CD3, Brilliant Violet 510™ anti-human CD3, Brilliant Violet 605™ anti-human CD3, Brilliant Violet 711™ anti-human CD3, Brilliant Violet 650™ anti-human CD3, APC/Fire™ 750 anti-human CD3, Brilliant Violet 785™ anti-human CD3, TotalSeq™-A0034 anti-human CD3, TotalSeq™-B0034 anti-human CD3, TotalSeq™-C0034 anti-human CD3, KIRAVIA Blue 520™ anti-human CD3, Spark Violet™ 538 anti-human CD3 Antibody, TotalSeq™-D0034 anti-human CD3, Spark Blue™ 574 anti-human CD3 Antibody, GMP Pacific Blue™ anti-human CD3, GMP PE anti-human CD3, GMP PE/Dazzle™ 594 anti-human CD3

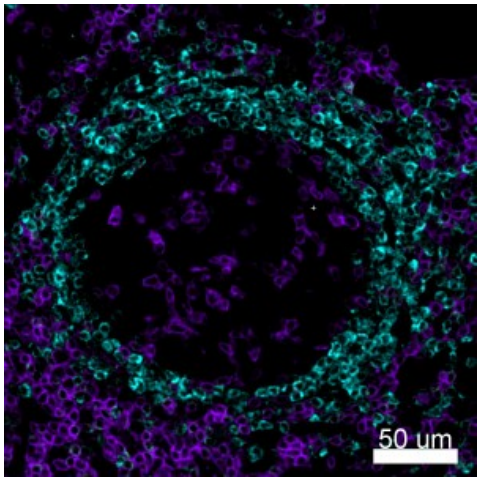
Product Data



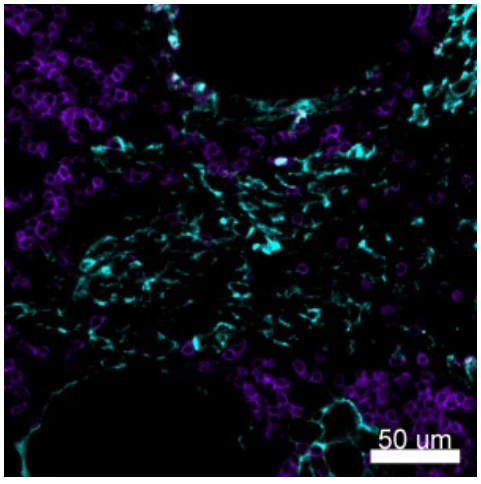
Human peripheral blood mononuclear cells were fixed with 2% paraformaldehyde (PFA), and then stained with 10 µg/ml CD14 (clone HCD14) Alexa Fluor® 647 (yellow), 10 µg/ml CD19 (clone HIB19) Alexa Fluor® 488 (green), and 10 µg/ml CD3 (clone UCHT1) Alexa Fluor® 594 (red) for 30 minutes at room temperature. Nuclei were counterstained with DAPI (blue). The image was captured with a 40X objective.



Human peripheral blood lymphocytes were stained with CD3 (clone UCHT1) Alexa Flour® 594 (filled histogram) or mouse IgG1, κ Alexa Flour® 594 isotype control (open histogram). The data was acquired by BD LSRFortessa™ cell analyzer equipped with the Yellow-Green Laser (561 nm).



Confocal image of human lymph node sample acquired using the IBEX method of highly multiplexed antibody-based imaging: BCL2 (cyan) in Cycle 1, CD3 (purple) 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).



Confocal image of human lymph node sample acquired using the IBEX method of highly multiplexed antibody-based imaging: CD3 (purple) in Cycle 1 and DC-SIGN (cyan) in Cycle 9. 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