Alexa Fluor® 647 anti-mouse CD4 Antibody

Catalog# / Size	100533 / 25 µg 100530 / 100 µg
Clone	RM4-5
Regulatory Status	RUO
Other Names	L3T4, T4
Isotype	Rat IgG2a, κ
Description	CD4 is a 55 kD protein also known as L3T4 or T4. It is a member of the Ig superfamily, primarily expressed on most thymocytes and a subset of T cells, and weakly on macrophages and dendritic cells. It acts as a co-receptor with the TCR during T cell activation and thymic differentiation by binding MHC class II and associating with the protein tyrosine kinase lck.

Product Details

Verified Reactivity	Mouse
Antibody Type	Monoclonal
Host Species	Rat
Immunogen	BALB/c mouse thymocytes
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* 3D IHC, IHC-F - *Verified*
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 10^6 cells in 100 µL volume. For 3D immunohistochemistry on formalin-fixed tissues, a concentration of 5.0 µg/mL is suggested. For immunohistochemistry on frozen tissue sections, a concentration range of 5.0 - 10 µg/mL is suggested. It is recommended that the reagent be titrated for optimal performance for each application. * Alexa Fluor® 647 has a maximum emission of 668 nm when it is excited at 633nm / 635nm. Alexa Fluor® and Pacific Blue™ are trademarks of Life Technologies Corporation. View full statement regarding label licenses
Excitation Laser	Red Laser (633 nm)
Application Notes	The RM4-5 antibody blocks the binding of GK1.5 antibody and H129.19 antibody to $CD4^+$ T cells, but not RM4-4 antibody. Additional reported applications (for the relevant formats) include: blocking of ligand binding, *in vivo* depletion of $CD4^+$ cells[1], and immunohistochemistry of acetone-fixed frozen tissue sections[2,3,11] and paraffin-embedded sections[11]. Clone RM4-5 is not recommended for immunohistochemistry of formalin-fixed paraffin sections. Instead, acetone frozen or zinc-fixed paraffin sections are recommended. The Ultra-LEAF™ Purified antibody (Endotoxin < 0.01 EU/µg, Azide-Free, 0.2 µm filtered) is recommended for functional assays (Cat. No. 100575 and 100576).
Application References **(PubMed link indicates**	1. Kruisbeek AM. 1991. *In Curr. Protocols Immunol.* pp. 4.1.1-4.1.5. (Block, Deplete) 2. Nitta H, *et al.* 1997. *Cell Vision* 4:73. (IHC)

BioLegend citation)

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Product Citations

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RRID

AB_493372 (BioLegend Cat. No. 100533)
AB_389325 (BioLegend Cat. No. 100530)

Antigen Details

Structure	Ig superfamily, 55 kD
Distribution	Majority of thymocytes, T cell subset
Function	TCR co-receptor, T cell activation
Ligand/Receptor	MHC class II molecule
Cell Type	Dendritic cells, T cells, Thymocytes, Tregs
Biology Area	Immunology
Molecular Family	CD Molecules
Antigen References	1. Barclay A, *et al.* 1997. The Leukocyte Antigen FactsBook Academic Press. 2. Bierer BE, *et al.* 1989. *Annu. Rev. Immunol.* 7:579. 3. Janeway CA. 1992. *Annu. Rev. Immunol.* 10:645.
Gene ID	12504

Related Protocols

Immunohistochemistry Protocol for Frozen Sections

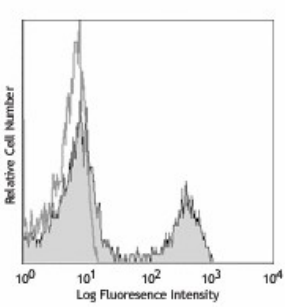
Cell Surface Flow Cytometry Staining Protocol

Ce3D™ Tissue Clearing Kit

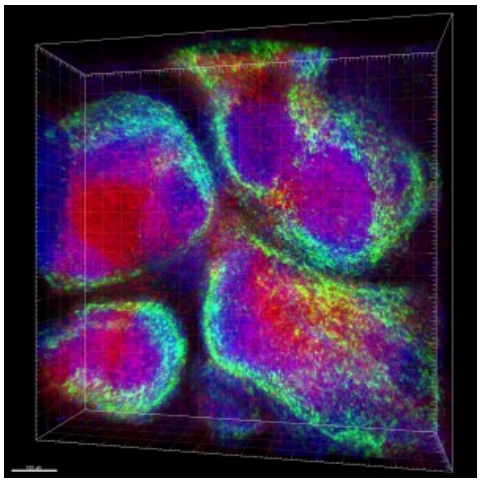
Other Formats

APC anti-mouse CD4, Biotin anti-mouse CD4, FITC anti-mouse CD4, PE anti-mouse CD4, PE/Cyanine5 anti-mouse CD4, Purified anti-mouse CD4, PE/Cyanine7 anti-mouse CD4, APC/Cyanine7 anti-mouse CD4, Alexa Fluor® 647 anti-mouse CD4, Alexa Fluor® 488 anti-mouse CD4, Pacific Blue™ anti-mouse CD4, Alexa Fluor® 700 anti-mouse CD4, PerCP anti-mouse CD4, PerCP/Cyanine5.5 anti-mouse CD4, Brilliant Violet 421™ anti-mouse CD4, APC/Fire™ 750 anti-mouse CD4, Brilliant Violet 570™ anti-mouse CD4, Brilliant Violet 605™ anti-mouse CD4, Brilliant Violet 650™ anti-mouse CD4, Brilliant Violet 711™ anti-mouse CD4, Brilliant Violet 785™ anti-mouse CD4, Brilliant Violet 510™ anti-mouse CD4, Purified anti-mouse CD4 (Maxpar® Ready), PE/Dazzle™ 594 anti-mouse CD4, TotalSeq™-A0001 anti-mouse CD4, TotalSeq™-B0001 anti-mouse CD4, TotalSeq™-C0001 anti-mouse CD4, Ultra-LEAF™ Purified anti-mouse CD4, Spark Violet™ 423 anti-mouse CD4 (L3T4) Antibody

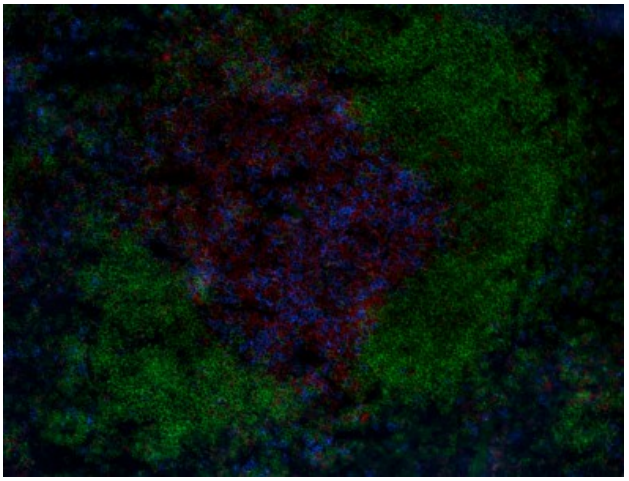
Product Data



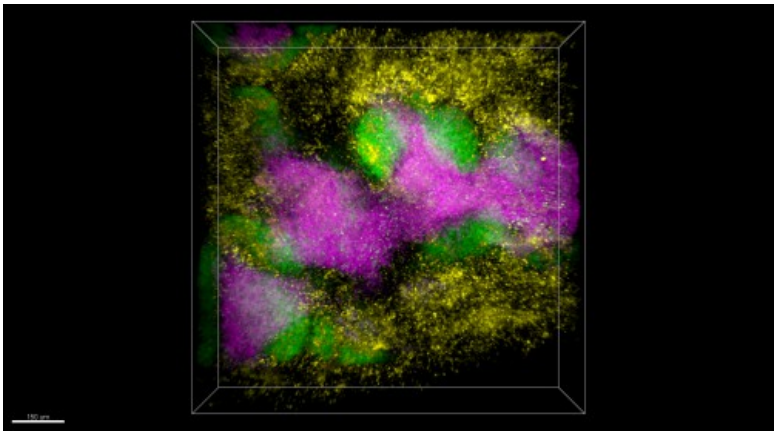
C57BL/6 mouse splenocytes stained with CD4 (clone RM4-5) Alexa Fluor® 647 (filled histogram) or rat IgG2a, κ Alexa Fluor® 647 isotype control (open histogram).



Formalin-fixed, 300 micron-thick mouse spleen section was blocked, permeabilized and stained overnight with CD4 (clone RM4-5) Alexa Fluor® 647 (red), CD169 (Siglec-1)(clone 3D6.112) Alexa Fluor® 488 (green), and CD45R/B220 (clone RA3-6B2) Alexa Fluor® 594 (blue) all at 5 µg/mL, optically cleared, then analyzed at 220 µm imaging depth on a confocal microscope. Watch the video.



C57BL/6 mouse frozen spleen section was fixed with 4% paraformaldehyde (PFA) for 10 minutes at room temperature and blocked with 5% FBS for 30 minutes at room temperature. Then the section was stained with 10 µg/mL of anti-mouse CD4 (clone RM4-5) Alexa Fluor® 647 (red), anti-mouse CD8b (clone YTS156.7.7) Brilliant Violet 421™ (blue) and anti-mouse B220 (clone RA3-6B2) Alexa Fluor® 488 (green) overnight at 4°C. The image was captured by 10X objective.



Paraformaldehyde-fixed (4%), 500 µm-thick mouse spleen tissue section was processed according to the Ce3DTM Tissue Clearing Kit protocol (cat. no. 427701). The section was costained with anti-mouse/human CD45R/B220 Antibody (clone RA3-6B2) Alexa Fluor® 488 at 5 µg/mL (green), anti-mouse CD18 Antibody (clone M18/2) Alexa Fluor® 594 at 5 µg/mL (yellow), and anti-mouse CD4 Antibody (clone RM4-5) 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.

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