Purified anti-LAT Phospho (Tyr171) Antibody

Catalog# / Size	946601 / 25 µL 946602 / 100 µL
Clone	A20005D
Regulatory Status	RUO
Other Names	Linker For Activation Of T Cells; P36-38
Isotype	Mouse IgG2b, κ
Description	LAT, or Linker for Activation of T cells, is a transmembrane protein expressed mainly in T cells and a limited number of other immune cells such as natural killer cells, mast cells, and immature B cells. As an adaptor protein, phosphorylated LAT facilitates the recruitment of other signaling proteins and functions as a nucleating site for multiprotein signaling complexes. These signaling complexes can propagate TCR, or T Cell antigen Receptors, and activate downstream effectors, which trigger T cell proliferation or cytokine expression. LAT (Tyr171) is one of the nine tyrosines in LAT conserved between human, mice, and rat. LAT (Tyr171) is necessary for the activation of PI 3-kinase, a protein that regulates the recruitment of other proteins that facilitate in T-cell receptor signal transduction such as PLC-gamma1 and Sos1. When LAT (Tyr171) and (Tyr191) is phosphorylated by ZAP-70, Gads can bind directly to LAT (Tyr171) and (Tyr191) and mediate the recruitment of SLP-76.

Product Details

Reactivity	Human, Mouse
Antibody Type	Monoclonal
Host Species	Mouse
Immunogen	Synthetic peptide corresponding to human LAT phosphorylated at Tyr 171
Formulation	Phosphate Buffer, 0.5% BSA, 0.09% NaN_3
Preparation	The antibody was purified by affinity chromatography.
Concentration	0.05 mg/mL
Storage & Handling	The antibody solution should be stored undiluted between 2°C and 8°C.
Application	WB - Quality tested ICC, ICFC - Verified
Recommended Usage	Each lot of this antibody is quality control tested by western blotting. For western blotting, the suggested dilution of this reagent is 1000 - 1:5000. For immunocytochemistry, a dilution of 1:10 is recommended. For intracellular flow cytometric staining, the suggested dilution of this reagent is 1:20 per million cells in 100 µL volume. It is recommended that the reagent be titrated for optimal performance for each application.
Application Notes	When this clone is used in WB at a dilution lower than 1:1000 it may show non-specific bands at 50 kD and above. This clone has been tested by ICC on H_2O_2 treated and untreated Jurkat, with three fix and permeabilization methods (100% methanol, 4% PFA plus methanol and 4% PFA plus Triton X-100). All three methods can be used for ICC staining, but the PFA plus Triton X-100 fixation/permeabilization method produced the best staining. This clone has only been tested on True-PhosTM perm buffer (Cat. No. 425401) by ICFC
RRID	AB_2904456 (BioLegend Cat. No. 946601) AB_2904456 (BioLegend Cat. No. 946602)

Antigen Details

Structure	LAT is a 262 amino acid protein with a predicted molecular weight of 36-38 kD.
Distribution	Plasma membrane/T Cells
Function	Plasma membrane/T Cells
Cell Type	Mast cells, NK cells, T cells
Biology Area	Cell Biology, Immunology, Signal Transduction
Molecular Family	Phospho-Proteins
Antigen References	1. Balagopalan L. *et al.,* 2010, *Cold Spring Harb Perspect Biol.,* 2(8): a005512 2. Balagopalan L. *et al.,* 2020, *PLoS One,* 15(2): e0229036 3. Lin J. and Weiss A., 2001, *J Biol Chem.,* 276(31): 29588-95 4. Paz *et. al.,* 2001, *Biochem J.*, 356, 461-71 5. Zhang W. *et al.,* 2000, *J Biol Chem.,* 275(30): 23355-61
Gene ID	27040

Related Protocols

Immunocytochemistry Staining Protocol

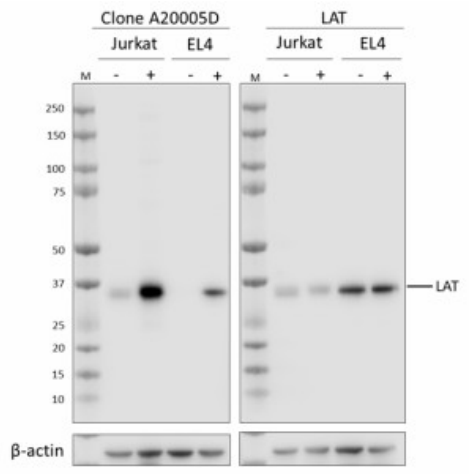
Western Blotting Protocol

Intracellular Flow Cytometry Staining Protocol

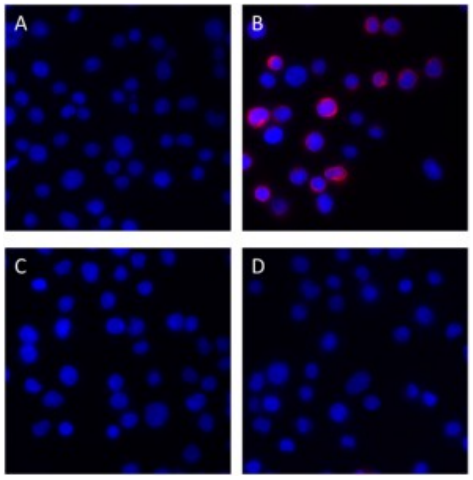
Other Formats

Purified anti-LAT Phospho (Tyr171)

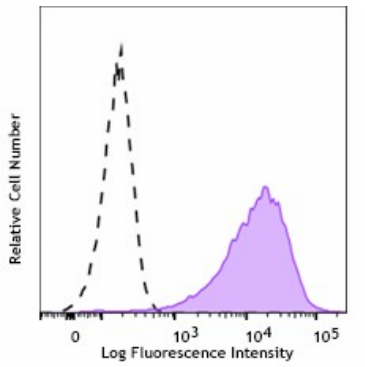
Product Data



Whole cell extracts (15 µg protein) from serum-starved Jurkat and EL4 cells untreated (-) or treated (+) with 5 mM H_2O_2 for 3 minutes, were resolved on a 4-12% Bis-Tris gel, transferred to a PVDF membrane, and probed with 0.0125 µg/mL (1:1000 dilution) of purified anti-LAT Phospho (Tyr171) (clone A20005D) for 2 hours at room temperature. Proteins were visualized by chemiluminescence detection using HRP goat anti-mouse IgG antibody (Cat. No. 405306) at a 1:3000 dilution. Equal LAT loading was confirmed by probing membranes with anti-LAT antibody at a 1:1000 dilution. Western-Ready™ ECL Substrate Premium Kit (Cat. No. 426319) was used as a detection agent. Direct-Blot™ HRP anti-β-actin antibody (Cat. No. 643807) was used as a loading control at a 1:10000 dilution (lower). Lane M: Molecular weight marker



Serum-starved Jurkat cells untreated (lower) and treated (upper) with 5 mM H_2O_2 for 3 minutes, were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with Triton X-100 for 10 minutes, and blocked with 5% FBS for 60 minutes. Cells were then intracellularly stained with 5.0 µg/mL of either purified mouse IgG2b, κ isotype control (Cat. No. 400302) (panel A and C) or purified anti-LAT Phospho (Tyr171) (clone A20005D) (panel B and D) (1:10 dilution) for 2 hours at room temperature, followed by incubation with Alexa Fluor® 594 goat anti-mouse IgG (Cat. No. 405326) at a 1:200 dilution. Nuclei were counterstained with DAPI and the image was captured with a 60X objective.



Jurkat cells treated (filled histogram, positive control) with 5mM of H_2O_2 for 3 min or untreated (open histogram, negative control) were fixed and permeabilized using the True-Phos™ Perm Buffer set (Cat. No. 425401), and intracellularly stained with 0.125 µg per test (1:20 dilution) purified anti-LAT Phospho (Tyr171) (clone A20005D) followed by PE goat anti-mouse IgG antibody (Cat. No. 405307).

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