Monoclonal Anti-human LAT-APC

Catalog Number: IC63341A
Lot Number: ABWD01
100 Tests

Reagents Provided

Allophycocyanin (APC)-conjugated mouse monoclonal anti-human LAT: Supplied as 10 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 661002

Isotype: mouse IgG_{2B}

Reagents Not Provided

Flow Cytometry Fixation Buffer (Catalog # FC004) or other 4% paraformaldehyde fixation buffer.

Flow Cytometry Permeabilization/Wash Buffer I (1X) (Catalog # FC005) or other saponin-containing saline buffer.

Storage

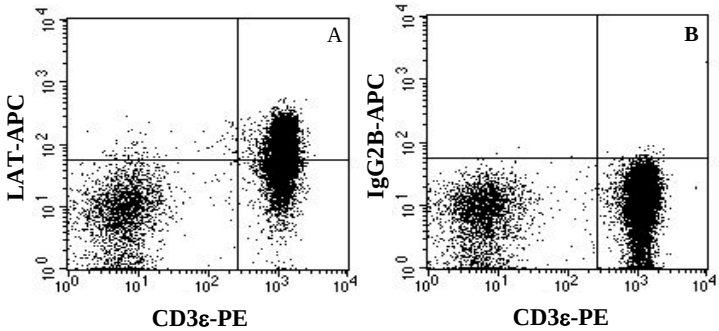
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

Intended Use

Designed to quantitatively determine the percentage of cells containing LAT within a population and qualitatively determine the density of intracellular LAT by flow cytometry.

Product Description

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with a synthetic phosphopeptide corresponding to the residues surrounding Y161 of human LAT (Accession # O43561). The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography. The purified antibody was then conjugated to APC fluorochrome. Intracellular expression of LAT is determined by flow cytometry using 620 - 650 nm wavelength excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 660 - 670 nm.



Whole blood lymphocytes were stained with A) APC-conjugated anti-human LAT (Catalog # IC63341A) or B) APC-conjugated isotype control (Catalog # IC0041A) and PE-conjugated anti-human CD3ε (Catalog # FAB100P).

Background Information

LAT (Linker for Activation of T cells) is a type III transmembrane lipid raft protein that serves as a scaffold for signaling molecules. Isoforms of 262 (36 kDa) and 233, called long and short, respectively, differ by inclusion/exclusion of aa 114 - 142; a 269 aa isoform lacks this region, but includes alternate N-terminal sequence. Upon T cell antigen receptor activation, LAT is multiply phosphorylated by ZAP-70/Syk protein tyrosine kinases, creating docking sites for SH2 domain-containing proteins. Phospholipase C-γ docks at Y161, which is p-Y132 in the short form of LAT. Mutation of this site results in a Th2 autoimmune lymphoproliferative disorder in mice.

Flow Cytometry Validation

For intracellular staining, cells must first be fixed and permeabilized. We recommend the use of 4% PFA as a fixative and a 0.1% saponin balanced salt solution for permeabilization and washing (see Reagents Not Provided).

1. Cells were harvested and washed twice in saline buffer.
2. Cell surface staining may be done at this point following the manufacturer's staining procedure.
3. Up to 1 x 10^6 cells were resuspended in 0.5 mL of cold Flow Cytometry Fixation Buffer (Catalog # FC004) and incubated at room temperature for 10 minutes.
4. Following fixation, the cells were washed twice in saline buffer, then once in Flow Cytometry Permeabilization/Wash Buffer I (Catalog # FC005).
5. After permeabilization, 10 μL of conjugated antibody was added and the cells were incubated for 30 minutes at room temperature **in the dark**.
6. The cells were washed twice with Flow Cytometry Permeabilization/Wash Buffer I.
7. The cells were resuspended in saline buffer for final flow cytometric analysis. As a control for this analysis, cells in a separate tube should be treated with APC-labeled mouse IgG_{2B} antibody. This procedure may need to be modified, depending on the cell type and final utilization. Individual users may need to titrate to determine the optimal reagent amount for their specific use.

Warning: Contains sodium azide as a preservative - sodium azide may react with lead and copper plumbing to form explosive metal azides. Flush with large volumes of water during disposal.

R&D Systems Inc.
1-800-343-7475