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ProductInformation

Anti-phospho-LAT [pTyr^{132}]
Developed in Rabbit, Affinity Isolated Antibody

Product Number **L 5292**

Product Description

Anti-phospho-LAT [pTyr^{132}] is developed in rabbit using a synthetic phosphorylated peptide derived from the region of human LAT that contains tyrosine 132 as immunogen. The antiserum is affinity purified using epitope-specific affinity chromatography. The antibody is preadsorbed to remove any reactivity toward a non-phosphorylated LAT peptide.

The antibody detects human LAT. Mouse and rat (92% homologous) LAT have not been tested, but are expected to react. It has been used in immunoblotting applications.

Linker for activation of T cells (LAT) is a 36 kDa transmembrane adapter protein that is tyrosine phosphorylated following T-cell receptor (TCR) stimulation by ZAP-70 and Syk. Four distal tyrosine residues (132, 171, 191 and 226) in human LAT are crucial for its activity and subsequent signaling to downstream molecules.

Tyrosine 132 mediates LAT binding to PLCγ-1, and is necessary for TCR-mediated calcium mobilization and activation of ERK and NFAT proteins.

Reagent

The antibody is supplied as a solution in Dulbecco's phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.3, with 1.0 mg/ml BSA (IgG and protease free) and 0.05% sodium azide

Precautions and Disclaimer

Due to the sodium azide content, a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

Storage/Stability

Store at −70 °C. Upon initial thawing freeze the solution in working aliquots for extended storage. Avoid repeated freezing and thawing to prevent denaturing the antibody. Do not store in frost-free freezers. Working dilution samples should be discarded if not used within 12 hours. The antibody is stable for at least 12 months when stored appropriately.

Product Profile

The supplied reagent is sufficient for 10 immunoblots.

A recommended working concentration of 0.1 to 1.0 µg/mL is determined by immunoblotting using Jurkat E6.1 cells

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working concentration by titration test.

Results

Peptide Competition

1. Extracts prepared from Jurkat E6.1 cells were left unstimulated (Lane 1) or stimulated (Lanes 2-5), and were resolved by SDS-PAGE on a 10% polyacrylamide gel and transferred to PVDF.
2. Membranes were blocked with a 5% BSA-TBST buffer overnight at 4 °C.
3. After blocking, membranes were preincubated with different peptides as follow:
   - Lane 1&2 no peptide
   - Lane 3 non phosphorylated peptide corresponding to the immunogen
   - Lane 4 a generic phosphotyrosine containing peptide
   - Lane 5 immunogen
1. After preincubation membranes were incubated with 0.50 µg/mL LAT [pTyr^{132}] antibody for two hours at room temperature in a 3% BSA-TBST buffer.
2. After washing, membranes were incubated with goat F(ab')$_2$ anti-rabbit IgG alkaline phosphatase and signals were detected.

The data in Figure 1 show that only the peptide corresponding to LAT [pTyr^{132}] blocks the antibody signal, demonstrating the specificity of the antibody.

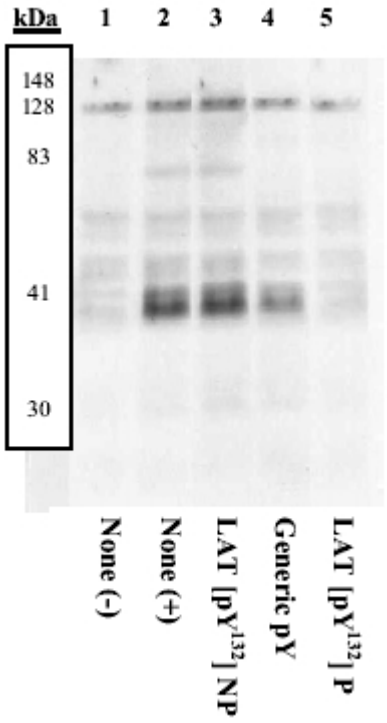

Figure 1 Peptide Competition

References

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