3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

Anti-phospho-SLP-76 [pTyr^{145}]
Developed in Rabbit, Affinity Isolated Antibody

Product Number **S 4569**

Product Description

Anti-phospho-SLP-76 [pTyr^{145}] is developed in rabbit using a synthetic phosphorylated peptide derived from the region of SLP-76 that contains tyrosine 145 as immunogen. The sequence is conserved in human, mouse, chicken and rat. The antiserum is affinity purified using epitope-specific affinity chromatography. The antibody is preadsorbed to remove any reactivity toward a non-phosphorylated SLP-76.

The antibody detects human SLP-76. Mouse and rat (85% homologous) SLP-76 have not been tested, but are expected to react. Chicken (62%) SLP-76 also has not been tested. It has been used in immunoblotting applications.

SLP-76 (SH2 domain–containing leukocyte protein of 76 kDa) is a hematopoietic cell-specific adaptor protein that is crucial for T-cell receptor (TCR) signaling, hemostasis and platelet function. TCR ligation and fibrinogen binding to integrin □II□3 stimulates the phosphorylation of the tyrosine residues in the amino terminus, and facilitates SLP-76 binding to the SH2 domain of Vav, which can activate JNK. SLP-76 also comprises a proline-rich domain region that associates with the SH3 domain of Grb2 linking SLP-76 to the Ras □Raf □ERK1&2 signaling pathway, LAT, PLC-γ, Fyn-binding protein (SLAP-130), the SH2-containing phosphatase-1 and Nck, which mediates the regulation of cytoskeletal actin polymerization.

Phosphorylation of tyrosine 145 has been shown to be important for optimal SLP-76 function.

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

One vial is sufficient for 10 immunoblots.

A recommended working concentration of 0.1 to 1.0 µg/mL is determined by immunoblotting using Jurkat cells +/- pervanadate treatment.

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. Jurkat cells endogenously expressing SLP-76 were serum starved and left untreated (Lane 1) or treated (Lanes 2-5) with 0.1 mM pervanadate .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
4. After preincubation membranes were incubated with 0.50 µg/mL SLP-76 [pTyr^{145}] antibody for two hours at room temperature in a 3% BSA-TBST buffer.
5. 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 SLP-76 [pTyr^{145}] blocks the antibody signal, thereby demonstrating the specificity of the antibody.

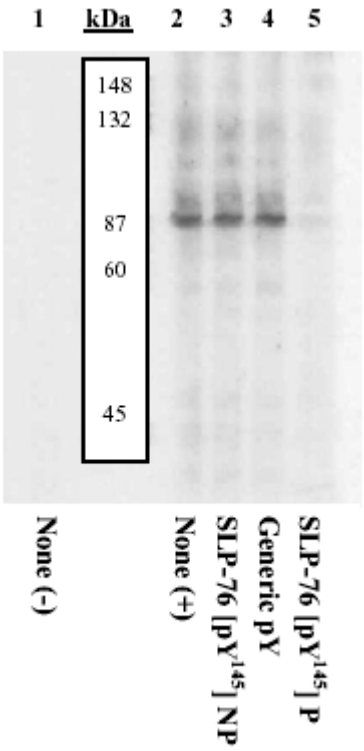

Figure 1 Peptide Competition

References

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