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-ZAP-70 [pTyr^{315}/pTyr^{319}]
produced in rabbit, affinity isolated antibody

Catalog Number **Z0276**

Product Description

Anti-phospho-ZAP-70 [pTyr^{315}/pTyr^{319}] is produced in rabbit using as immunogen a synthetic phosphorylated peptide derived from the region of human ZAP-70 that contains tyrosines 315 and 319. The antiserum is affinity purified using epitope-specific affinity chromatography. The antibody is preadsorbed to remove any reactivity toward a non-phosphorylated ZAP-70 peptide.

The antibody detects human ZAP-70 by immuno-blotting. Mouse ZAP-70 (100% homologous) has not been tested, but is expected to react.

ZAP-70 (Zeta-Associated Protein) is a 70 kDa member of the Syk tyrosine kinase family that plays a central role in lymphocyte activation and development, and is implicated in several immune disorders. Upon T-cell antigen receptor (TCR) engagement, ZAP-70 is phosphorylated on tyrosines 292, 315 and 319 in the interdomain B, located between the SH2 and kinase domains.

Phosphorylation of tyrosine 315 and 319 enhances ZAP-70 function in mediating lymphocyte signaling, while tyrosine 292 terminates transient activation and attenuates lymphocyte signaling. Phosphorylation of tyrosines 315 and 319 plays an important role in mediating the positive and negative selection of T cells in thymus.

Reagent

Supplied as a solution in Dulbecco's phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.3, with 50% glycerol, 1.0 mg/ml BSA (IgG and protease free) and 0.05% sodium azide.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store at –20 °C. Due to the presence of 50% glycerol the antibody will remain in solution. For extended storage, centrifuge the vial briefly before opening and prepare working aliquots. The antibody is stable for at least six months when stored appropriately. Working dilutions should be discarded if not used within 12 hours.

Product Profile

Immunoblotting: a recommended working dilution of 1:1000 is determined using Jurkat cells treated with H_2O_2.

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

Results

Peptide Competition

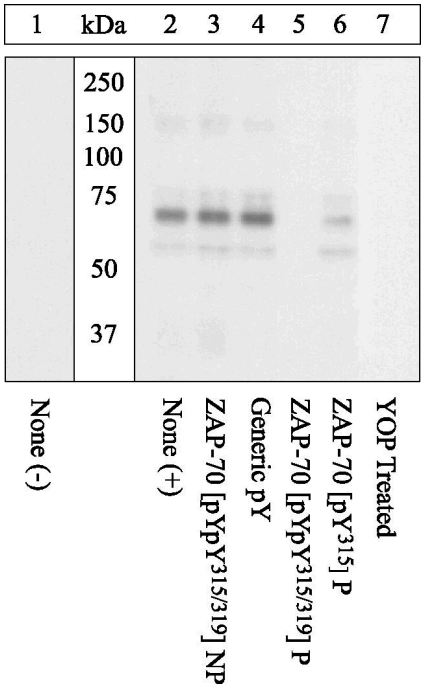
1. Lysates prepared from Jurkat cells left untreated (Lane 1) or treated with H_2O_2 (Lanes 2-7) were resolved by SDS-PAGE on a 10% polyacrylamide gel and transferred to PVDF.
2. Membranes were blocked with a 5% BSA-TBST buffer for one hour at room temperature.
3. After blocking, membranes were preincubated with different peptides as follows:

Lane 1, 2	no peptide
Lane 3	non phosphorylated peptide corresponding to the immunogen
Lane 4	a generic phosphotyrosine containing peptide
Lane 5	phosphopeptide immunogen
Lane 6	ZAP-70 [pTyr^{315}]
Lane 7	YOP phosphatase treated

4. After preincubation membranes were incubated with 0.50 µg/mL ZAP [pTyr^{315}/pTyr^{319}] 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-HRP and signals were detected.

The data show that only the peptide corresponding to ZAP-70 [pTyr^{315}/pTyr^{319}] completely blocks the antibody signal. The data also show that phosphatase stripping eliminates the signal, verifying that the antibody is phospho-specific.

Peptide Competition



References

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