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^{292}]
Developed in Rabbit, Affinity Isolated Antibody

Product Number **Z 0151**

Product Description

Anti-phospho-ZAP-70 [pTyr^{292}] (Zeta-associated protein) is developed in rabbit using a synthetic phosphorylated peptide derived from the region of human ZAP-70 that contains tyrosine 292 as immunogen. 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. Mouse ZAP-70 (100% homologous) has not been tested, but is expected to react. The antibody has been used in immunoblotting applications.

Zeta-associated protein (ZAP-70), a 70 kDa member of the Syk tyrosine kinase family, 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.

ZAP-70 tyrosine 292 is the negative regulatory site that modulates the duration of activated TCR at the cell surface, and functions as a docking site for Cbl.

Reagent

Anti-phospho-ZAP-70 [pTyr^{292}] is 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

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 –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

A recommended working dilution of 1:1000 is determined by immunoblotting 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 2-6) 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, 6 no peptide
   - Lane 2 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 ZAP-70 [pTyr^{292}] 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 show that only the peptide corresponding to ZAP-70 [pTyr^{292}] blocks the antibody signal. The data also show that phosphatase stripping eliminates the signal, verifying that the antibody is phospho-specific.

References

1. Orchard, J.A., et al., ZAP-70 expression and prognosis in chronic lymphocytic leukaemia. Lancet. **363**, 105-111(2004).
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3. Bottini, N., et al., Activation of ZAP-70 through specific dephosphorylation at the inhibitory Tyr-292 by the low molecular weight phosphotyrosine phosphatase (LMPTP). J. Biol. Chem., **277**, 24220-24224 (2002).
4. Magnan, A., et al. T cell development and T cell responses in mice with mutations affecting tyrosines 292 or 315 of the ZAP-70 protein tyrosine kinase. J. Exp. Med. **194**, 491-505 (2001).

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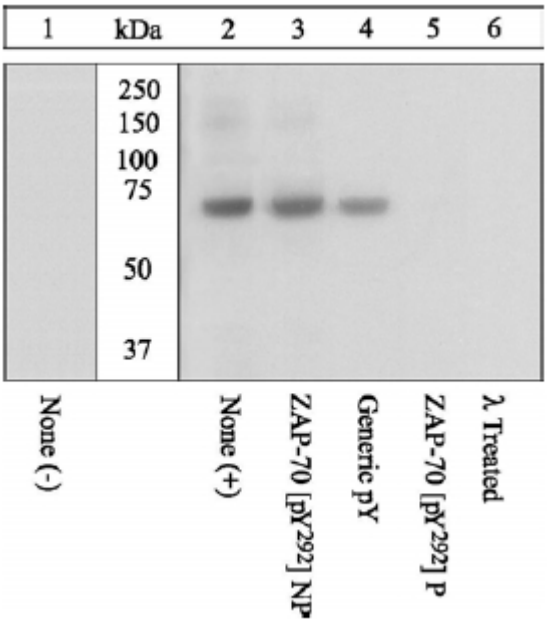

Figure 1 Peptide Competition