

Product Specification

ZAP70, active

(Full-length recombinant protein enzyme expressed in Sf 9 cells)

Catalog # : 7752
 Lot # : _____
 Aliquot size: 5 µg protein in 50 µl
 Specific activity: 113 nmol/min/mg

Quality Control Analysis

Activity assessment

ZAP70 protein (~100 ng/µl concentration) was diluted to 25ng/µl with assay dilution buffer (4 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 10 mM MnCl₂, 4 mM MgCl₂, 1 mM EGTA, 0.05 mM DTT), followed by 2-fold serial dilutions, and then the 10µl diluted proteins were used to phosphorylate the Poly(Glu-Tyr, 4:1) substrate in the following assay condition:

- 10 µl diluted ZAP70 protein
- 10 µl Poly(Glu-Tyr) substrate (1 mg/ml stock)
- 5 µl [³²P] ATP mixture (250 □M ATP, 0.16 µCi/µl in 4x assay dilution buffer)

The various reaction components, except [³²P] ATP, were incubated at 30°C and the reaction started by the addition of [³²P] ATP. After 15 minutes, the reaction was terminated by spotting 20 µl of the reaction mixture onto a phosphocellulose P81 paper. The P81 paper was dried and washed several times in 1% phosphoric acid prior to counting in the presence of scintillation fluid in a scintillation counter. The actual counts, using various dilutions of the enzyme in the assay, are shown in Fig. 1.

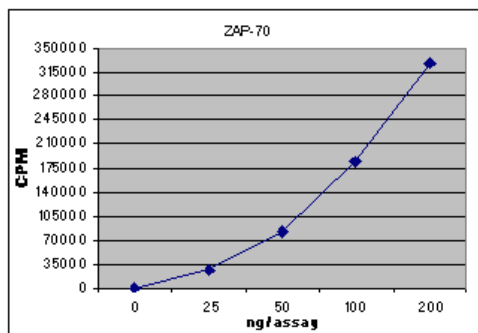


Fig. 1 ZAP70 activity assay

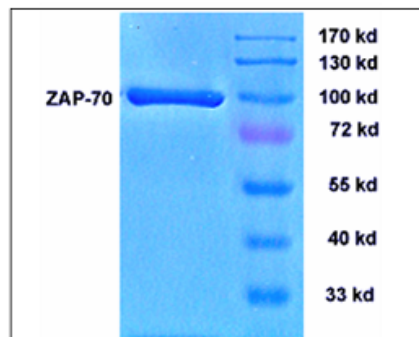


Fig. 2 ZAP70 protein gel

Purity assessment

3 µg of ZAP70 protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the gel showed >90% purity of the ZAP70 products, and the band was at ~96 kDa (Fig. 2).

Product Description

Recombinant N-terminal GST tag, full length human ZAP-70 was expressed by baculovirus in Sf9 insect cells.

The gene accession number is NM_001079.

This material is sold for research purposes only.

Specific Activity

113 nmol phosphate incorporated into Poly(Glu-Tyr) substrate per minute per mg protein at 30°C for 15 minutes using a final concentration of 50 μ M ATP (0.83 μ Ci/assay in 25ul reaction volume).

Formulation

Recombinant protein in storage buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, 25% glycerol).

Storage and Stability

Store product frozen at or below -70°C. Stable for 1 year at -70°C as undiluted stock. Aliquot to avoid repeated thawing and freezing.

Scientific Background

ZAP70 is a non-receptor protein tyrosine kinase (part of the Syk/Zap70 family) that is involved in signaling by the T-cell antigen receptor (TCR). Ligation of the TCR/CD3 receptor in Jurkat T-cells induces phosphoprotein complexes which contain ZAP70 (1). TCR zeta chains are initially phosphorylated by p56Lck that lead to the recruitment of ZAP70 via its SH2 domain. ZAP70 in turn phosphorylates other proteins in the TCR-phosphoprotein complex. ZAP70 tyrosine kinase is tyrosine phosphorylated in Jurkat T cells and in purified peripheral T cells after MHC-I ligation. The phosphorylation of ZAP70 after MHC-I ligation is dependent on TCR/CD3 surface expression. One of the natural substrates for ZAP70 is the zeta-chain dimer of the TCR/CD3 complex (2). Another substrate of ZAP70 is LAT (linker for activation of T cells). Direct tyrosine phosphorylation of LAT with activated protein-tyrosine kinase Zap70 is necessary and sufficient for the association and activation of signalling proteins. Zap-70 efficiently phosphorylates LAT on tyrosine residues at positions 226, 191, 171, 132 and 127. By substituting these tyrosine residues in LAT with phenylalanine and by utilizing phosphorylated peptides derived from these sites, the tyrosine residues in LAT have been shown to be required for the direct interaction and activation of Vav, p85/p110alpha and phospholipase Cgamma1 (PLCgamma1) (3).

References

1. Nel AE, Gupta S, Lee L, Ledbetter JA, Kanner SB. Ligation of the T-cell antigen receptor (TCR) induces association of hSos1, ZAP-70, phospholipase C-gamma 1, and other phosphoproteins with Grb2 and the zeta-chain of the TCR. *J Biol Chem.* 1995 Aug 4;270(31):18428-36.
2. Skov S, Bregenholt S, Claesson MH. MHC class I ligation of human T cells activates the ZAP70 and p56lck tyrosine kinases, leads to an alternative phenotype of the TCR/CD3 zeta-chain, and induces apoptosis. *J Immunol.* 1997 Apr 1;158(7):3189-96.
3. Paz PE, Wang S, Clarke H, Lu X, Stokoe D, Abo A. Mapping the Zap-70 phosphorylation sites on LAT (linker for activation of T cells) required for recruitment and activation of signalling proteins in T cells. *Biochem J.* 2001 Jun 1;356(Pt 2):461-71.