

## ZAP70 (Human), Active

Full-length recombinant protein expressed in Sf9 cells

Cat# CY-SPZ02

Lot No. B108-1  
5 µg 0.1 µg/µl

### 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. One of the natural substrates for ZAP70 is the zeta-chain dimer of the TCR/CD3 complex (2).

### Product Description:

Recombinant full-length human ZAP70 was expressed by baculovirus in Sf9 insect cells using a N-terminal GST tag. The gene accession number is NM\_001079.

### Gene Aliases:

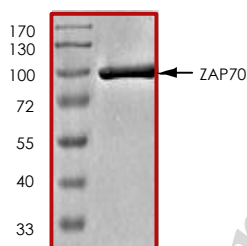
SRK; STD; ZAP-70

### Formulation:

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

### Purity & Molecular Weight:

The purity was determined to be >95% by densitometry. Approx. MW 96kDa.



### Storage:

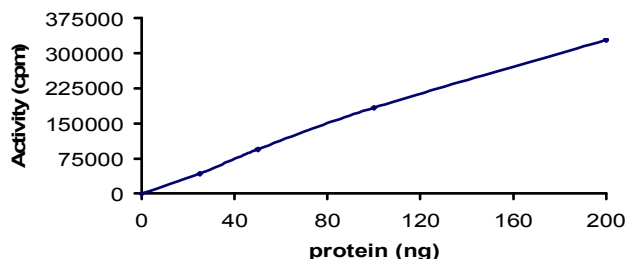
Store product at  $-70^{\circ}\text{C}$ . For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

### Stability:

Unopened vial at  $-70^{\circ}\text{C}$ , 1 year from date of shipment.

**Specific Activity:**

The specific activity was determined to be 113 nmol/min/mg as per Activity Assay Protocol.

**Activity Assay Protocol:**

Assay activity of the kinase in a 25  $\mu$ L reaction consisting of 5  $\mu$ L of 5 X Kinase Assay Buffer, 10  $\mu$ L of 1 mg/ml the Substrate Solution, 5  $\mu$ L of diluted kinase and 5  $\mu$ L of 250  $\mu$ M ATP solution containing [ $\gamma$ - $^{32}$ P] ATP (0.167  $\mu$ Ci/ $\mu$ L). Start the reaction by adding the ATP solution. Incubate for 15 minutes at 30°C. Terminate the reaction by spotting 20  $\mu$ L of the reaction mixture onto phosphocellulose P81 paper. Air-dry the P81 paper and sequentially wash 4 times for approximately 10 minutes each in 1% phosphoric acid with constant gentle stirring. Count the P81 paper in a liquid scintillation counter.

**Substrate Solution:**

Poly (Glu:Tyr, 4:1) synthetic peptide substrate diluted in distilled H<sub>2</sub>O to a final concentration of 1 mg/ml.

**5 X Kinase Assay Buffer:**

25mM MOPS, 12.5mM  $\beta$ -glycerol-phosphate, 25mM MgCl<sub>2</sub>, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

**References:**

1. Duplay, P. et al: p56lck interacts via its src homology 2 domain with the ZAP-70 kinase. J Exp Med. 1994 Apr 1;179(4):1163-72.
2. Chan, A C. et al: Differential expression of ZAP-70 and Syk protein tyrosine kinases, and the role of this family of protein tyrosine kinases in TCR signaling. J Immunol. 1994 May 15;152(10):4758-66.

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