Recombinant Human Active ZAP70, CF

Catalog Number: 3709-KS-010
Lot Number: 1779664

SPECIFICATIONS AND USE

Source	Recombinant full-length human ZAP70 was expressed by baculovirus in *Sf*9 insect cells using an N-terminal GST tag. The gene accession number is NM_001079.
Gene Aliases	SRK; STD; ZAP-70
Molecular Mass	The approximate molecular weight is 96 kDa.
Purity	The purity was determined to be > 90% by densitometry.
Formulation	Supplied in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 mM Glutathione, 0.1 mM EDTA, 0.25 mM DTT, 0.1 mM PMSF, 25% Glycerol.
Size	10 µg
Concentration	0.1 µg/µL
Activity	The specific activity of ZAP70 was determined to be 13 nmol/min/mg as per Activity Assay Protocol.
Storage	This product is stable at ≤ -70 °C for up to one year from the date of receipt. For optimal storage, aliquot into smaller quantities after centrifugation and store at recommended temperature. **Avoid repeated freeze-thaw cycles.**

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).

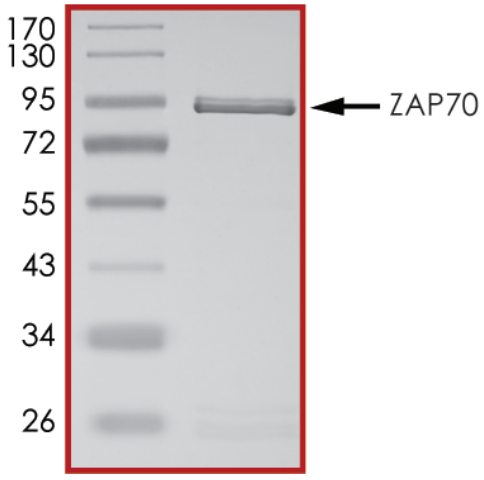

Figure 1: The approximate molecular weight is 96 kDa and the purity is > 90%.

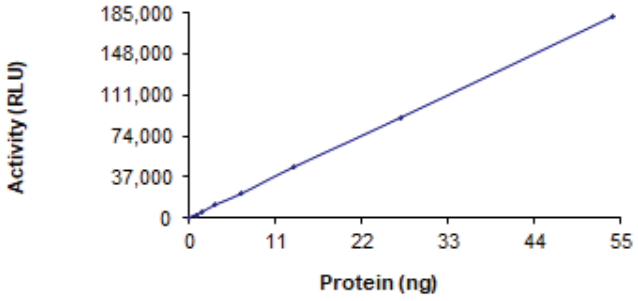

Figure 2: The specific activity of ZAP70 was determined to be 13 nmol/min/mg as per Activity Assay Protocol.

REFERENCES

1. Duplay, P. *et al.* (1994) J. Exp. Med. **179**:1163.
2. Chan, A.C. *et al.* (1994) J. Immunol. **152**:4758.

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ACTIVITY ASSAY PROTOCOL

Solutions Required

Active Kinase - (0.1 µg/µL) diluted with Kinase Dilution Buffer X and assayed as outlined in sample activity plot.
Note: *These are suggested working dilutions. Optimal dilutions should be determined by each laboratory for each application.*

Kinase Assay Buffer III (5X) - 200 mM Tris-HCl, pH 7.4, 100 mM $MgCl_2$, and 0.5 mg/mL BSA. Add fresh DTT prior to use to a final concentration of 250 µM.

Kinase Dilution Buffer X - 40 mM Tris-HCl, pH 7.4, 20 mM $MgCl_2$, 2.5 mM $MnCl_2$, and 0.1 mg/mL BSA. Add fresh DTT prior to use to a final concentration of 50 µM.

ADP-Glo™ Kinase Assay Kit - 10 mM of ATP Solution, 10 mM of ADP Solution, ADP-Glo™ Reagent, Kinase Detection Reagent.

Substrate - Poly (4:1 Glu, Tyr) synthetic peptide substrate diluted 25 mM Tris-HCl (pH 7.5) solution to a final concentration of 1 mg/mL.

Cofactor: 2.5M $MnCl_2$ - Diluted in distilled water to a working concentration of 1M.

ASSAY PROCEDURE

The ZAP70 assay is performed using the ADP-Glo™ Kinase Assay Kit which quantifies the amount of ADP produced by the ZAP70 reaction. The ADP- Glo™ Reagent is added to terminate the kinase reaction and to deplete the remaining ATP, and then the Kinase Detection Reagent is added to convert ADP to ATP and to measure the newly synthesized ATP using luciferase/luciferin reaction.

1. Thaw the Active ZAP70, Kinase Assay Buffer III (5X), and Substrate on ice. Prepare a 15 µL enzyme dilution with Kinase Dilution Buffer X at the desired concentration, in a pre-chilled 96-well plate.
2. Prepare a substrate/ATP mixture as follows (25 µM ATP example):

Reaction Component	Amount
10 mM ATP Solution	1 µL
Kinase Assay Buffer III (5X)	78 µL
Substrate at 1 mg/mL	80 µL
1M $MnCl_2$	1 µL

3. Transfer the following reaction components prepared in steps 1 and 2 to a 384-well opaque plate, bringing the reaction volume up to 5 µL:

Component 1	3 µL of diluted Active ZAP70
Component 2	2 µL of Substrate/ATP mix as prepared in the table above. This initiates the reaction.

4. Set up the blank control as outlined in step 2, excluding the addition of the kinase. Replace the kinase with an equal volume of Kinase Dilution Buffer X.
5. Incubate at ambient temperature for 40 minutes.
6. After the 40-minute incubation period, terminate the reaction and deplete the remaining ATP by adding 5 µL of ADP-Glo™ Reagent. Spin down and shake the 384-well plate. Then incubate the reaction mixture for another 40 minutes at ambient temperature.
7. Then add 10 µL of the Kinase Detection Reagent to the 384-well plate and incubate the reaction mixture for another 30 minutes at ambient temperature.
8. Read the 384-well reaction plate using the Luminescence Module Protocol on a GloMax®-Multi Microplate Multimode Reader.
9. Determine the corrected activity (RLU) by removing the blank control value (see step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of Specific Activity of ADP (RLU/pmol)
From ADP standard curve, determine RLU/pmol of ADP

Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)
Corrected RLU from reaction / [(SA of ADP in RLU/pmol) * (Reaction time in min) * (Enzyme amount in µg or mg)