Recombinant Human Active SYK Protein

Catalog Number: 4594-KS-010

Lot Number: 1797859

SPECIFICATIONS AND USE

Source	Recombinant human SYK was expressed by baculovirus in *Sf* 9 insect cells using an N-terminal GST tag. The gene accession number is NM_003177.
Molecular Mass	The approximate molecular weight is 102 kDa.
Purity	The purity was determined to be > 75% by densitometry.
Formulation	Supplied in 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.25 mM DTT, 0.1 mM EDTA, 10 mM Glutathione, 0.1 mM PMSF, and 25% Glycerol.
Size	10 µg
Concentration	0.1 µg/µL
Activity	The specific activity of SYK was determined to be 67 nmol/min/mg using a synthetic peptide substrate.
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

SYK is a non-receptor protein tyrosine kinase that is widely expressed in hematopoietic cells. It is involved in coupling activated immunoreceptors to downstream signaling events that mediate diverse cellular responses, including proliferation, differentiation, and phagocytosis. In B cells, SYK plays a crucial role in intracellular signal transduction induced by oxidative stress as well as antigen receptor engagement (1). SYK has been shown to act as a potential tumor suppressor in breast cancer. Absence of SYK protein in primary breast tumors is correlated with poor outcomes. SYK deficient cells have increased motility that is restored to normalcy by replacement with wild-type SYK (2).

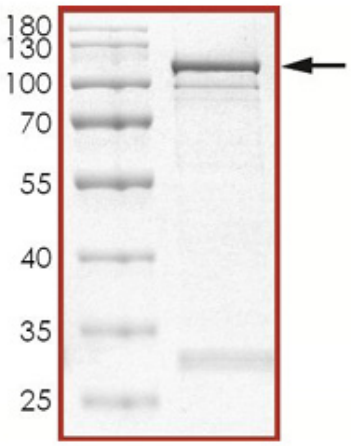

Figure 1: The approximate molecular weight is 102 kDa and the purity is > 75%.

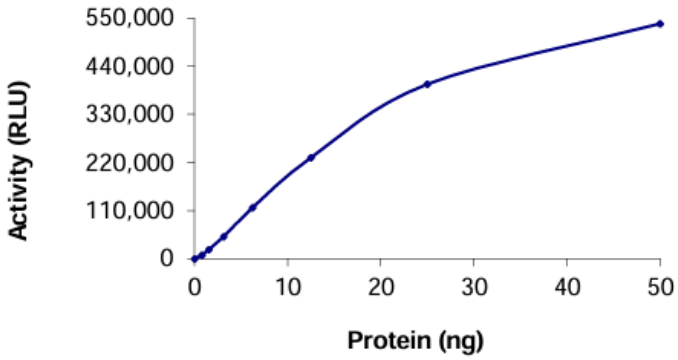

Figure 2: The specific activity of this lot of SYK was determined to be 67 nmol/min/mg as per the Activity Assay Protocol.

REFERENCES

1. Takano, T. *et al*. (2002) Antioxid. Redox. Signal. **4**:533.
2. Navara, C.S. (2004) Curr. Pharm. Des. **10**:1739.

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ACTIVITY ASSAY PROTOCOL

Solutions Required

Active Kinase - Active SYK (0.1 µg/µL) diluted with Kinase Dilution Buffer X as shown in Figure 2.
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 (1X) - 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 ATP Solution, 10 mM ADP Solution, ADP-Glo™ Reagent, Kinase Detection Reagent.

Substrate - Poly (Glu:Tyr, 4:1) synthetic peptide substrate diluted in distilled water to a final concentration of 1 mg/mL.

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

ASSAY PROCEDURE

The SYK assay is performed using the ADP-Glo™ Kinase Assay Kit which quantifies the amount of ADP produced by the SYK 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 SYK, Kinase Assay Buffer lll (5X), and Substrate on ice. Prepare a 15 µL enzyme dilution at the desired concentration, with Kinase Dilution Buffer X (1X), in a pre-chilled 96-well plate.
2. Prepare a substrate/ATP mixture as follows (25 µM example):

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

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

Component 1	3 µL of diluted Active SYK
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 IX (1X).
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. 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 ATP-ADP conversion 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)]