SYK, Active
Full-length recombinant protein expressed in Sf9 cells

Catalog # S52-10G
Lot # K1562-3

Product Description

Recombinant full-length human SYK was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_003177.

Gene Aliases

N/A

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, 25% glycerol.

Storage and Stability

Store product at –70°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.

Scientific 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).

References


To place your order, please contact us by phone 1-(604)-232-4600, fax 1-604-232-4601 or by email: orders@signalchem.com
www.signalchem.com

FOR IN VITRO RESEARCH PURPOSES ONLY. NOT INTENDED FOR USE IN HUMAN OR ANIMALS.
**Activity Assay Protocol**

**Reaction Components**

**Active Kinase (Catalog #: S52-10G)**

Active SYK (0.1µg/µl) diluted with Kinase Dilution Buffer IV (Catalog #: K24-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active SYK for optimal results).

**Kinase Dilution Buffer IV (Catalog #: K24-09)**

Kinase Assay Buffer II (Catalog #: K02-09) diluted at a 1:4 ratio (5X dilution) with 50 ng/µl BSA solution.

**Kinase Assay Buffer II (Catalog #: K02-09)**

Buffer components: 25mM MOPS pH 7.2, 12.5mM β-glycerol-phosphate, 20mM MgCl2, 12.5mM MnCl2, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

**[33P]-ATP Assay Cocktail**

Prepare 250µM [33P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [33P]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer II (Catalog #: K02-09). Store 1ml aliquots at –20°C.

**10mM ATP Stock Solution (Catalog #: A50-09)**

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of distilled H2O to a final concentration of 1mg/ml.

**Substrate (Catalog #: P61-58)**

Poly (Glu4,Tyr1) synthetic peptide substrate diluted in distilled H2O to a final concentration of 1mg/ml.

**Assay Protocol**

Step 1. Thaw [33P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.

Step 2. Thaw the Active SYK, Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.

Step 3. In a pre-cooled microtube tube, add the following reaction components bringing the initial reaction volume up to 20µl:

- Component 1. 10µl of diluted Active SYK (Catalog #S52-10G)
- Component 2. 5µl of 1 mg/ml stock solution of substrate (Catalog #P61-58)
- Component 3. 5µl distilled H2O (4°C)

Step 4. Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H2O.

Step 5. Initiate the reaction by the addition of 5µl [33P]-ATP Assay Cocktail bringing the final volume up to 25µl and incubate the mixture in a water bath at 30°C for 15 minutes.

Step 6. After the 15 minute incubation period, terminate the reaction by spotting 20µl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.

Step 7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H2O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.

Step 8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.

Step 9. Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

**Calculation of [P33]-ATP Specific Activity (SA) (cpm/pmole)**

Specific activity (SA) = cpm for 5µl [33P]-ATP / pmoles of ATP (in 5µl of a 250µM ATP stock solution, i.e., 1250 pmoles)

**Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)**

Corrected cpm from reaction / [(SA of 33P-ATP in cpm/pmole) *(Reaction time in min) *(Enzyme amount in µg or mg)] / [(Reaction Volume) / (Spot Volume)]

---

To place your order, please contact us by phone 1-(604)-232-4600, fax 1-604-232-4601 or by email: orders@signalchem.com

**FOR IN VITRO RESEARCH PURPOSES ONLY. NOT INTENDED FOR USE IN HUMAN OR ANIMALS.**