

# Syk Kinase

✓ 5 µg

**Orders** ■ 877-616-CELL (2355)  
orders@cellsignal.com  
**Support** ■ 877-678-TECH (8324)  
info@cellsignal.com  
**Web** ■ www.cellsignal.com

New 03/07

This product is for *in vitro* research use only and is not intended for use in humans or animals.

**Description:** Purified recombinant full-length human Syk (Met1-Asn635) kinase, supplied as a GST fusion protein.

**Background:** Syk, a protein tyrosine kinase, is widely expressed and plays an important role in intracellular signal transduction in hematopoietic cells (1-3). Syk interacts with immunoreceptor tyrosine-based activation motifs (ITAMs) located in the cytoplasmic domains of immune receptors (4). It couples the activated immunoreceptors to downstream signaling events that mediate diverse cellular responses, including proliferation, differentiation and phagocytosis (4). There is also evidence of a role for Syk in nonimmune cells, and Syk is a potential tumor suppressor in human breast carcinomas (5). Tyr323 is a negative regulatory phosphorylation site within the SH2-kinase linker region in Syk. Phosphorylation of Tyr323 provides a direct binding site to the TKB domain of Cbl (6,7). Tyrosine 352 of Syk is involved in the association of PLC-γ1 (8). Tyrosines 525 and 526 are located in the activation loop of the Syk kinase domain, and phosphorylation of Tyr525/526 of human Syk (equivalent to the Tyr519/520 of mouse Syk) is essential for Syk function (9).

**Source/Purification:** The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing full-length human Syk (Met1-Asn635) (GenBank Accession No. NM\_003177) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using glutathione-agarose.

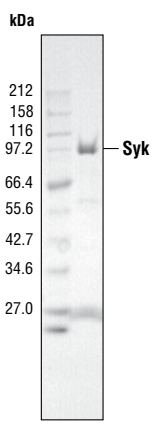


Figure 1. The purity of the GST-Syk fusion protein was analyzed using SDS/PAGE followed by Coomassie stain.

**Quality Control:** The theoretical molecular weight of the GST-Syk fusion protein is 100 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Coomassie stain [Fig.1]. Syk kinase activity was determined using a radiometric assay [Fig.2].

**Background References:**

- (1) Cheng, A.M. and Chan, A.C. (1997) *Curr. Opin. Immunol.* 9, 528-533.
- (2) Kurosaki, T. et al. (1997) *Curr. Opin. Immunol.* 9, 309-318.
- (3) Chu, D.H. et al. (1998) *Immunol. Rev.* 165, 167-180.
- (4) Turner, M. et al. (2000) *Immunol. Today* 21, 148-154.
- (5) Coopman, P.J. et al. (2000) *Nature* 406, 742-747.
- (6) Decker, M. et al. (1998) *J. Biol. Chem.* 273, 8867-8874.
- (7) Rao, N. et al. (2001) *EMBO J.* 20, 7085-7095.
- (8) Law, C.L. et al. (1996) *Mol. Cell. Biol.* 16, 1305-1315.
- (9) Zhang, J. et al. (2000) *J. Biol. Chem.* 275, 35442-35447.

**Storage:** Enzyme is supplied in 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, 7 mM glutathione. Store at -80°C.

Keep on ice during use.  
Avoid repeated freeze-thaw cycles.

- Companion Products:**  
Tyrosine Kinase Substrate Screening Kit #7450  
HTScan® Tyrosine Kinase Buffer (4X) #9805  
ATP (10 mM) #9804  
HTScan® Syk Kinase Assay Kit #7779  
Gastrin Precursor (Tyr87) Biotinylated Peptide #1310  
Phospho-Tyrosine Mouse mAb (P-Tyr-100) #9411

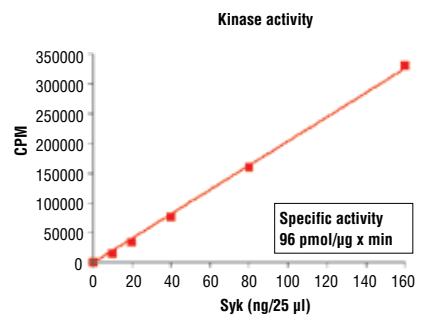


Figure 2. Syk kinase activity was measured in a radiometric assay using the following reaction conditions: 5 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 4 mM MgCl<sub>2</sub>, 2.5 mM MnCl<sub>2</sub>, 0.05 mM DTT, 50 µM ATP, Substrate: Poly EY, 400 ng/µL, and recombinant Syk: variable.

## Protocol for Syk Kinase Assay

**Note:** Lot-specific information for this kinase is provided on the enzyme vial. Optimal assay incubation times and enzyme concentrations must be determined empirically for each lot of kinase under specified conditions.

### A Additional Solutions and Reagents (Not included)

- 1. Kinase Buffer (10X)**  
50 mM MOPS, pH 7.2  
25 mM  $\beta$ -glycerophosphate  
10 mM EGTA  
4 mM EDTA  
50 mM  $MgCl_2$   
25 mM  $MnCl_2$   
0.5 mM DTT
- 2. ATP (10 mM) #9804**
- 3.  $^{32}P$ - $\gamma$ ATP**
- 4. Poly EY (Glu:Tyr 4:1, 1  $\mu$ g/ $\mu$ l)**

### B Suggested Protocol

- 1.** Dilute 10 mM ATP with 3X assay buffer 1:40 to make 250  $\mu$ M ATP.
- 2.** Dilute [ $^{32}P$ ] ATP to 0.16  $\mu$ Ci/ $\mu$ l [ $^{32}P$ ] ATP with 250  $\mu$ M ATP solution.
- 3.** Transfer enzyme from -80°C to ice. Allow enzyme to thaw on ice.
- 4.** Dilute Syk protein to 16 ng/ $\mu$ l with 1X assay buffer followed by 2-fold serial dilutions.
- 5.** To start the reaction combine 10  $\mu$ l diluted Syk kinase solution, 10  $\mu$ l Poly EY (1  $\mu$ g/ $\mu$ l), and 5  $\mu$ l 0.16  $\mu$ Ci/ $\mu$ l [ $^{32}P$ ] ATP solution.

### Final Assay Conditions

- 5 mM MOPS, pH 7.2
  - 2.5 mM  $\beta$ -glycerophosphate
  - 1 mM EGTA
  - 0.4 mM EDTA
  - 4 mM  $MgCl_2$
  - 2.5 mM  $MnCl_2$
  - 0.05 mM DTT
  - 400 ng/ $\mu$ L Poly EY
- 6.** After 15 minutes terminate reaction by spotting 20  $\mu$ l of the reaction mixture onto phosphocellulose P81 paper.
  - 7.** Air dry the P81 paper then wash with 1% phosphoric acid 3 times.
  - 8.** Transfer P81 paper to 4 ml scintillation tube then add 3 ml scintillation cocktail.
  - 9.** Count samples in a scintillation counter.

Cell Signaling Technology offers a full line of protein kinases, substrates, and antibody detection reagents for high throughput screening. Please direct all inquiries to: [drugdiscovery@cellsignal.com](mailto:drugdiscovery@cellsignal.com).