Recombinant Human Active SYK

Certificate of Analysis

Catalog Number: 4594-KS
Lot Number: 1444656

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 100 kDa (see Figure 1 below).
Purity	♦ The purity was determined to be > 90% by densitometry (see Figure 1 below).
Formulation	♦ Supplied in 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.25 mM DTT, 10 mM glutathione, 0.1 mM EDTA, 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 107 nmol/min/mg using a poly (Glu:Tyr, 4:1) synthetic peptide substrate (see 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.**

SYK

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

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

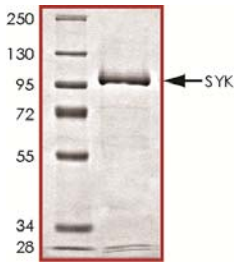

Figure 1: The approximate molecular weight is 100 kDa and the purity is > 90%.

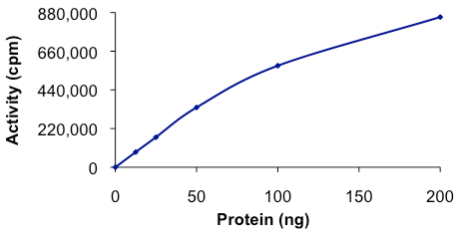

Figure 2: The specific activity of this lot of SYK was determined to be 107 nmol/min/mg as per the Activity Assay Protocol (on reverse).

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Activity Assay Protocol

Solutions Required

- **Active Kinase** - Active SYK (0.1 μg/μL) diluted with Kinase Dilution Buffer. **Note:** *These are suggested working dilutions. Optimal dilutions should be determined by each laboratory for each application.*
- **Kinase Assay Buffer II, pH 7.2** - 25 mM MOPS, 12.5 mM β-glycerolphosphate, 20 mM $MgCl_2$, 12.5 mM $MnCl_2$, 5 mM EGTA, 2 mM EDTA. Add 0.25 mM DTT to the Kinase Assay Buffer prior to use.
- **Kinase Dilution Buffer, pH 7.2** - Kinase Assay Buffer II diluted 5-fold with a 50 ng/μL BSA solution.
- **10 mM ATP Stock Solution** - Prepare the ATP Stock Solution by dissolving 55 mg of ATP in 10 mL of Kinase Assay Buffer II. Store 200 μL aliquots at ≤ -20° C.
- **[^{33}P]-ATP Assay Cocktail** - Prepare 250 μM [^{33}P]-ATP Assay Cocktail in a designated radioactive work area by combining 150 μL of 10 mM ATP Stock Solution, 100 μL of [^{33}P]-ATP (1 mCi/100 μL), and 5.75 mL of Kinase Assay Buffer II. Store 1 mL aliquots at ≤ -20° C.
- **Substrate** - Poly (Glu:Tyr, 4:1) synthetic peptide diluted in distilled or deionized water to a final concentration of 1 mg/mL.

Assay Procedure

1. Thaw the [^{33}P]-ATP Assay Cocktail in a shielded container in a designated radioactive work area.
2. Thaw the Active SYK, Kinase Assay Buffer II, Substrate, and Kinase Dilution Buffer on ice.
3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20 μL.

Reaction Component	Amount
Diluted Active SYK	10 μL
Poly Substrate (1 mg/mL; on ice)	5 μL
Distilled or deionized water (on ice)	5 μL

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 or deionized water.
5. Initiate the reaction with the addition of 5 μL [^{33}P]-ATP Assay Cocktail, bringing the final volume up to 25 μL. Incubate the mixture in a water bath at 30° C for 15 minutes.
6. After the 15 minute incubation, terminate the reaction by spotting 20 μL of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (add 10 mL of phosphoric acid to 990 mL of distilled or deionized water) with constant gentle stirring. It is recommended that the strips be washed a total of three times for approximately 10 minutes each.
8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
9. Determine the corrected cpm by subtracting the blank control value (see step 4) for each sample and calculate the kinase specific activity as outlined below.

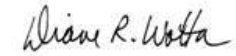
Calculation of [^{33}P]-ATP Specific Activity (SA) (cpm/pmol)

Specific Activity (SA) = cpm for 5 μL [^{33}P]-ATP/pmole of ATP (in 5 μL of a 250 μM ATP stock solution, *i.e.* 1250 pmoles)

Calculation of Kinase Specific Activity (SA) (pmol/minutes/μg or nmol/minutes/mg)

Corrected cpm from reaction / [(SA of ^{33}P-ATP in cpm/pmol) x (Reaction time in minutes) x (Enzyme amount in μg or mg)] x [(Reaction volume) / (Spot Volume)]

Diane Wotta



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