Recombinant RSV Active Src (v-Src)

Certificate of Analysis

Catalog Number: 3389-KS
Lot Number: 1475429

Specifications and Use

Source	♦ Recombinant full-length Rous sarcoma virus Src was expressed by baculovirus in *Sf* 9 insect cells using an N-terminal GST tag. The gene accession number is M11753.
Molecular Mass	♦ The approximate molecular weight is 83 kDa (see Figure 1 below).
Purity	♦ The purity was determined to be > 95% by densitometry (see Figure 1 below).
Formulation	♦ Supplied in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.25 mM DTT, 0.1 mM EDTA, 0.1 mM PMSF, and 25% glycerol.
Size	♦ 10 μg.
Concentration	♦ 0.1 μg/μL.
Activity	♦ The specific activity of Src was determined to be 112 nmol/min/mg using a synthetic peptide substrate (KVEKIGEGTYGVVYK) (see activity assay protocol).
Storage	♦ This product is stable at ≤ -70 °C for up to 1 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.**

Src

The non-receptor tyrosine kinase Src was originally identified as a transforming protein of the Rous sarcoma virus that had enzymatic ability to phosphorylate tyrosine in protein substrates (1). The proto-oncogene c-Src is the cellular homologue of viral Src (v-Src) and the founding member of the Src family kinases. c-Src is over-expressed and activated in a large number of human maligancies and has been linked to the development of cancer and progression to distant metastases (2). In addition to increasing cell proliferation, a key role of c-Src in cancer seems to be the ability to promote invasion and motility, functions that might contribute to tumor progression.

Although v-Src and c-Src share 88% amino acid identity, v-Src, unlike c-Src, is constitutively active mainly because it lacks a crucial c-terminal negative-regulatory region (3). As a result, v-Src is missing a tyrosine residue (Y530 in human c-Src) that upon phosphorylation contributes to c-Src assuming an inactive conformation.

References

1. Collett, M.S. *et al*. (1978) Proc. Natl. Acad. Sci. USA **75**:2021.
2. Jacobs, C. *et al*. (1983) Cancer Res. **43**:1696.
3. Yeatman, T.J. (2004) Nature Reviews Cancer **4**:470.

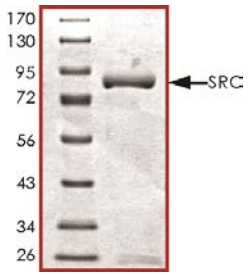

Figure 1: SDS-PAGE and Coomassie® stain. The approximate molecular weight is 83 kDa and the purity is > 95%.

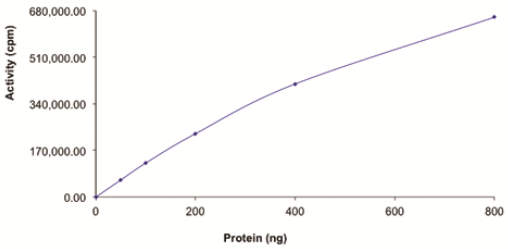

Figure 2: Enzymatic assay results. The specific activity of Src was determined to be 112 nmol/min/mg as per activity assay protocol (on reverse).

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Activity Assay Protocol

Solutions Required

- **Active Kinase** - Active Src (0.1 μg/μL) diluted with Kinase Dilution Buffer IV and assayed as outlined in Figure 2.
  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, 5 mM EGTA, 2 mM EDTA. Add 0.25 mM DTT to the Kinase Assay Buffer prior to use.
- **Kinase Dilution Buffer IV, pH 7.2** - Kinase Assay Buffer II diluted at a 1:4 ratio (5-fold dilution) with 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 I.
- **[^{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 I.
- **Substrate** - Src synthetic peptide substrate (KVEKIGEGTYGVVYK) diluted in distilled or deionized water to a final concentration of 1.0 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 Src, Kinase Assay Buffer I, Substrate, and Kinase Dilution Buffer IV 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 Src	10 μL
Substrate (1 mg/mL Stock Solution)	5 μL
Distilled H_2O	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 by 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 period, 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 (dilute 10 mL of phosphoric acid and make a 1 liter solution with 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 removing 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/min/μg or nmol/min/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)]

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