Recombinant Viral RSV Active Src Protein, CF

Catalog Number: 3389-KS-010
Lot Number: 1757170

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.
Purity	The purity was determined to be > 95% by densitometry.
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).
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

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.

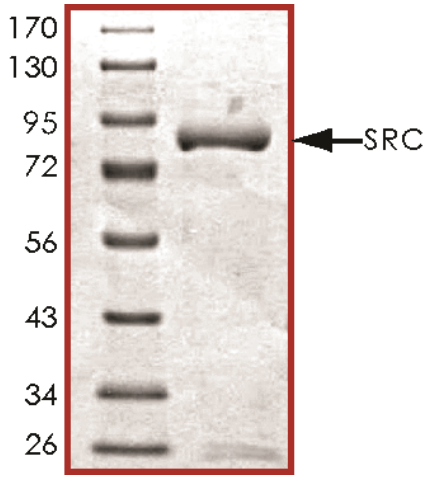

Figure 1: The approximate molecular weight is 83 kDa and the purity is > 95%.

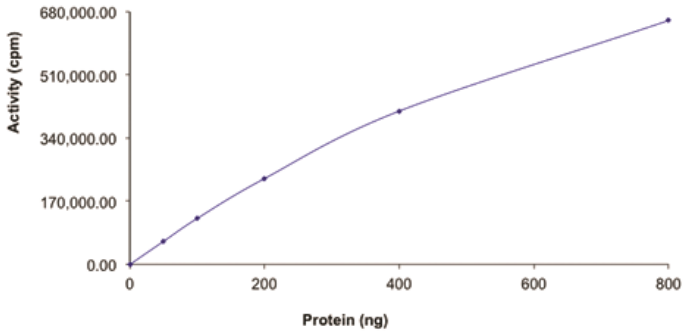

Figure 2: The specific activity of Src was determined to be 112 nmol/min/mg as per Activity Assay Protocol.

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.

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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 - 25 mM MOPS, pH 7.2, 12.5 mM β-glycerolphosphate, 20 mM $MgCl_2$, 12.5 mM $MnCl_2$, 5 mM EGTA, 2 mM EDTA. Add fresh DTT prior to use to a final concentration of 250 µM prior to use.

Kinase Dilution Buffer IV - Kinase Assay Buffer II diluted at a 1:4 ratio (5X 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 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 - Src synthetic peptide substrate (KVEKIGEGTYGVVYK) diluted in distilled 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 Src, Kinase Assay Buffer II, 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 at 1 mg/mL	5 µL
Distilled (2-8 °C)	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 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 (add 10 mL of phosphoric acid to 990 mL and make a 1 liter solution with distilled 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 (step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of [33P]-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)]