

Product Specification

Src, active

(Full-length recombinant protein expressed in Sf 9 cells)

Catalog #: 7750-5
 Lot #: _____
 Aliquot size: 5µg protein in 50 µl
 Specific activity: 116 nmol/min/mg

Quality Control Analysis

Activity assessment

Src protein (~100 ng/µl concentration) was diluted to 20ng/µl with assay dilution buffer (4 mM MOPS, pH 7.2, 2.5 mM β -glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 2.5 mM MnCl₂, 4 mM MgCl₂, 0.05 mM DTT), followed by 2-fold serial dilutions, and then the 10µl diluted proteins were used to phosphorylate the Src substrate peptide (KVEKIGEGTYGVVYK) in the following assay condition:

- 10µl diluted Src protein
- 10 µl Src substrate peptide (1 mg/ml stock)
- 5 µl [³²P] ATP mixture (250 µM ATP, 0.16 µCi/µl in 4x assay dilution buffer)

The various reaction components, except [³²P] ATP, were incubated at 30⁰C and the reaction started by the addition of [³²P] ATP. After 15 minutes, the reaction was terminated by spotting 20 µl of the reaction mixture onto a phosphocellulose P81 paper. The P81 paper was dried and washed several times in 1% phosphoric acid prior to counting in the presence of scintillation fluid in a scintillation counter. The actual counts, using various dilutions of the enzyme in the assay, are shown in Fig. 1.

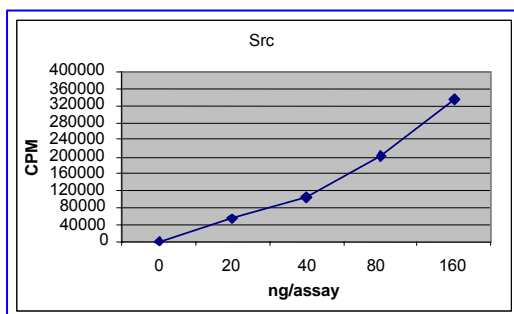


Fig. 1 Src 1 activity assay

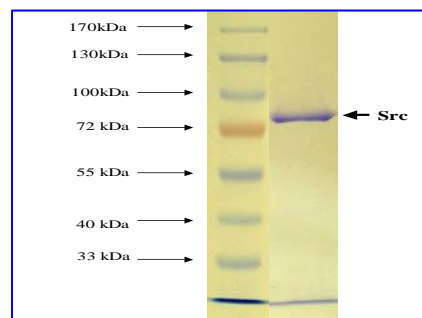


Fig. 2 Src1 protein gel

Purity assessment

2 µg of Src protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the gel showed >90% purity of the Src 1 product, and the band was at ~85 kDa (Fig. 2).

Product Description

Recombinant full length Rous sarcoma virus Src protein containing N-terminal GST tag was expressed by baculovirus in Sf9 insect cells.

The gene accession number is M11753.

This material is sold for research purposes only.

Specific Activity

116 nmol phosphate incorporated into Src substrate peptide per minute per mg proteins at 30°C for 15 minutes using a final concentration of 50 μM ATP (0.83 μCi/assay in 25ul reaction volume).

Formulation

Recombinant protein in storage buffer (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).

Storage and Stability

Store product frozen at or below -70°C. Stable for 1 year at -70°C as undiluted stock. Aliquot to avoid repeated thawing and freezing.

Scientific Background

Src family belongs to non-receptor tyrosine kinases. Src was originally identified as a transforming protein of the Rous sarcoma virus (RSV) that had enzymatic ability to phosphorylate tyrosine in protein substrates. Src is overexpressed and activated in a large number of human malignancies and has been linked to the development of cancer and progression to distant metastases. In addition to increasing cell proliferation, a key role of Src in cancer seems to be the ability to promote invasion and motility, functions that might contribute to tumour progression. Oncogenic forms of the Src alter cell structure, in particular the actin cytoskeleton and the adhesion networks that control cell migration, and also transmit signals that regulate proliferation and cell survival. Recent work indicates that Src plays a role in these functions by influencing the RhoA-ROCK pathway that controls contractile actin filament assembly, the STAT family of transcription factors needed for transformation, and the Cbl ubiquitin ligase that controls Src protein levels. These studies also shed light on the role of focal adhesion kinase (FAK) downstream of v-Src and other signaling pathways in controlling migration, invasion and survival of transformed cells. Src directly phosphorylates integrins and can also modulate R-Ras activity. Moreover, it stimulates the E-cadherin regulator Hakai, interacts with and phosphorylates the novel podosome-linked adaptor protein Fish, and progressively phosphorylates the gap junction component connexin 43. In addition to the above functions and substrates, the role in cellular physiology of Src is continually expanding.