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# SRC, Active

Full-length recombinant protein expressed in Sf9 cells

**Catalog # S19-10G**
*Lot # B176-1*

## Product Description

Recombinant full-length Rous sarcoma virus SRC was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is M11753.

## Gene Aliases

ASV; SRC1; v-SRC; p60-Src

## Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 0.25mM DTT, 0.1mM EGTA, 0.1mM EDTA, 0.1mM PMSF, 25% glycerol.

## Storage and Stability

Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

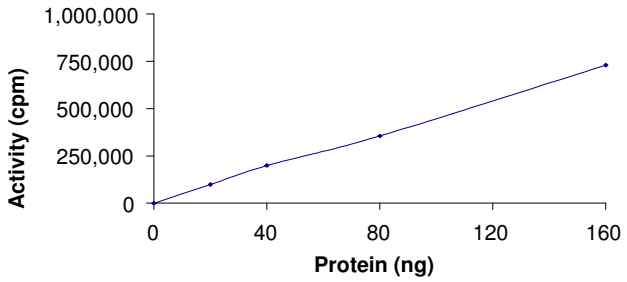
## Scientific Background

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 (1). 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 (2). 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

## References

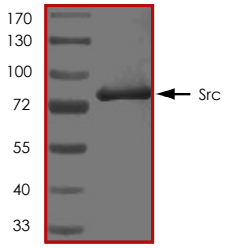
1. Collett, M S. et al: Protein kinase activity associated with the avian sarcoma virus src gene product. Proc Natl Acad Sci U S A. 1978 Apr;75(4):2021-4.
2. Jacobs, C. et al: Expression of pp60c-src protein kinase in adult and fetal human tissue: high activities in some sarcomas and mammary carcinomas. Cancer Res. 1983 Apr;43(4):1696-702.

## Specific Activity




The specific activity of SRC was determined to be **116 nmol /min/mg** as per activity assay protocol.

## Purity




The purity was determined to be **>90%** by densitometry.
Approx. MW **85kDa**.

# SRC, Active

Full-length recombinant protein expressed in Sf9 cells

| | |
|---|---|
| Catalog Number | S19-10G |
| Specific Activity | 116 nmol/min/mg |
| Specific Lot Number | B176-1 |
| Purity | >90% |
| Concentration | 0.1µg/µl |
| Stability | 1yr at –70°C from date of shipment |
| Storage & Shipping | Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice. |

# Activity Assay Protocol

## Reaction Components

**Active Kinase** (Catalog #: S19-10G)

Active SRC (0.1μg/μl) diluted with Kinase Dilution Buffer II (Catalog #: K22-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active SRC for optimal results).

**Kinase Dilution Buffer II** (Catalog #: K22-09)

Kinase Assay Buffer II (Catalog #: K02-09) diluted at a 1:4 ratio (5X dilution) with distilled $H_2O$.

**Kinase Assay Buffer II** (Catalog #: K02-09)

Buffer components: 25mM MOPS, pH 7.2, 12.5mM β-glycerol-phosphate, 20mM $MgC1_2$, 25mM $MnC1_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

**[$^{32}$P]-ATP Assay Cocktail**

Prepare 250μM [$^{32}$P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150μl of 10mM ATP Stock Solution (Catalog #: A50-09), 100μl [$^{32}$P]-ATP (1mCi/100μl), 5.75ml of Kinase Assay Buffer II (Catalog #: K02-09). Store 1ml aliquots at –20°C.

**10mM ATP Stock Solution** (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer II (Catalog #: K02-09). Store 200μl aliquots at –20°C.

**Substrate** (Catalog #: S30-58)

SRC synthetic peptide substrate (KVEKIGEGTYGVVYK) diluted in distilled $H_2O$ to a final concentration of 1mg/ml.

## Assay Protocol

**Step 1.** Thaw [$^{32}$P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.

**Step 2.** Thaw the Active SRC, Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.

**Step 3.** In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20μl:

- **Component 1.** 10μl of diluted Active SRC (Catalog #S19-10G)
- **Component 2.** 10μl of 1mg/ml stock solution of substrate (Catalog #S30-58)

**Step 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 $H_2O$.

**Step 5.** Initiate the reaction by the addition of 5μl [$^{32}$P]-ATP Assay Cocktail bringing the final volume up to 25μl and incubate the mixture in a water bath at 30°C for 15 minutes.

**Step 6.** After the 15 minute incubation period, terminate the reaction by spotting 20μl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.

**Step 7.** Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled $H_2O$) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.

**Step 8.** Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.

**Step 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 [P$^{32}$]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5μl [$^{32}$P]-ATP / pmoles of ATP (in 5μl of a 250μM ATP stock solution, i.e., 1250 pmoles)

## Kinase Specific Activity (SA) (pmol/min/μg or nmol/min/mg)

Corrected cpm from reaction / [(SA of $^{32}$P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in μg or mg)]*[(Reaction Volume) / (Spot Volume)]

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