

Certificate of Analysis

Src (1–530), active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-746, 14-746-K, 14-746M

Parent Lot # 0610042668

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: N-terminal 6His-tagged, recombinant, human Src, amino acids 1–530, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA-agarose.

The mutant form of Src (Src, 1–530) has been identified in cases of advanced colon cancer. This truncating mutation generates an open active form of Src that has been found to be transforming, tumorigenic and metastasis promoting.

Purity 100% by SDS-PAGE and Coomassie blue staining. MW = 61.1kDa

Formulation: 2.265mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

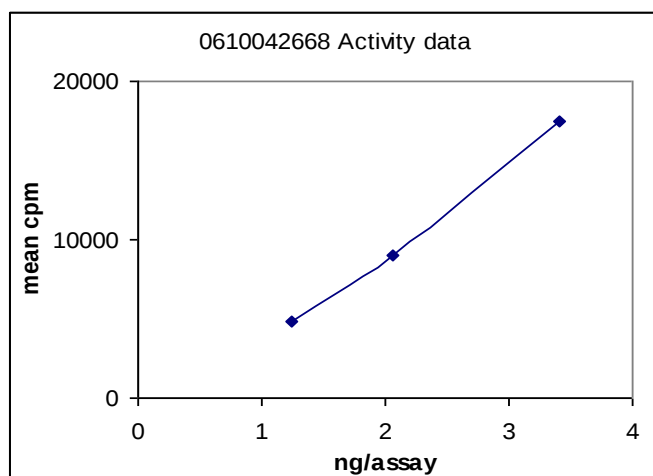
Specific Activity (Parent lot# 0610042668): 1956U/mg, where one unit of Src (1–530), active activity is defined as 1nmol phosphate incorporated into 500μM (GGEEEEYFELVKKKK) per minute at 30°C with a final ATP concentration of 100μM.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

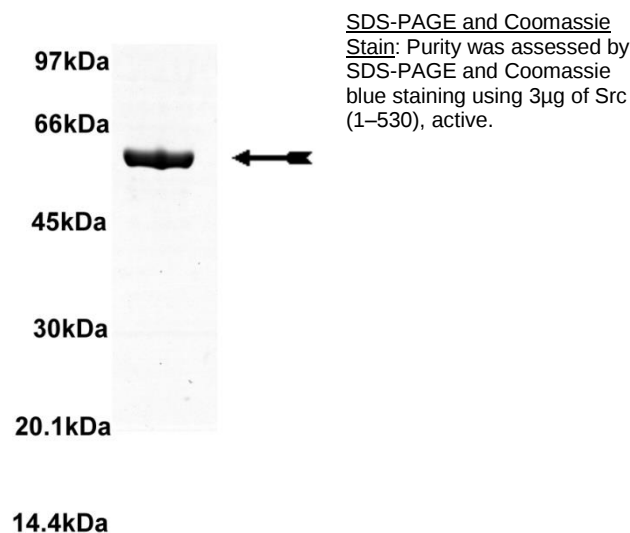
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 1.2–3.4ng of this lot of enzyme phosphorylated 50μM (GGEEEEYFELVKKKK) in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS Tryptic Fingerprint: Confirmed identity as Src (1–530) with the translated native sequence listed on page three.



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS-NaOH pH7.0, 1mM EDTA.
2. **(GGEEEEYFELVKKKK):** Use at a final concentration of 500 μ M. Make up a 5mM stock. Add 2.5 μ l of stock per assay point.
3. **Src (1–530), active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 1.2–3.4ng per assay point.
4. **[γ -³³P]ATP:** 2.5 x magnesium acetate/[γ -³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ -³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 μ l of 500 μ M (GGEEEEYFELVKKKK).
3. Add 2.5 μ l (1.2–3.4ng) Src (1–530), active.
4. Add 5 μ l of dH₂O.
5. Add 10 μ l of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
8. Transfer a 10 μ l aliquot onto the appropriate area of a **P30 Filtermat**.
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1 μ l of 30% phosphoric acid.

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Src (1–530) Sequence Information

Protein	human Src (1–530)
Tags	N-terminal 6His
Native sequence	M14 of the recombinant protein is equivalent to M1 of native human Src
Accession number	EMBL K03218

Recombinant Src (1–530) amino acid sequence:

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1 MSYYHHHHHH DEFMGSNKS PKDASQRRRS LEPAENVHGA GGAFFPASQT PSKPASADGH
61 RGPSAAFAPA AAEPKLFGGF NSSDTVTSPQ RAGPLAGGVT TFVALYDYES RTETDLSFKK
121 GERLQIVNNT EGDWWLAHSL STGQTGYIPS NYVAPSDSIQ AEEWYFGKIT RRERERLLL
181 AENPRGTFLV RESETTKGAY CLSVSDFDNA KGLNVKHYKI RKLDSSGGFYI TSRTQFNSLQ
241 QLVAYYSKHA DGLCHRLTTV CPTSKPQTQG LAKDAWEIPR ESLRLEVKLG QGCFGEVWMG
301 TWNGTTRVAI KTLKPGTMSP EAFLQEAQVM KKLREHEKLVQ LYAVVSEEPY YIVTEYMSKG
361 SLIDFLKGET GKYLRLPQLV DMAAQIASGM AYVERMNYVH RDLRAANILV GENLVCKQAD
421 FGLARLIEDN EYTRQGAKE PIKWTAPAAA LYGRFTIKSD VWSFGILLTE LTTKGRVPYP
481 GMVNREVLQD VERGYRMPCP PECPESLHDL MCQCWRKEPE ERPTFEYLQA FLEDYFTSTE
541 PQY

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Recombinant Src (1-530) nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gatgaattca tgggtagcaa caagagcaag
61 cccaaggatg ccagccagcg gcgcccgcgc ctggagcccg ccgagaacgt gcacggcgct
121 ggcggggggc ctttccccgc ctgcagagcc cccagcaagc cagcctcggc cgacggccac
181 cgcggcccca gcgcggcctt cgccccgcgc gccgcccagc ccaagctgtt cggaggcttc
241 aactcctcgg acaccgtcac ctccccgcag agggcgggccc cgctggccgg tggagtgacc
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1501 ccggagtgtc ccgagtcctt gcacgacctc atgtgccagt gctggcggaa ggagcctgag
1561 gagcgcccca cttcgagta cctgcaagcc ttctggagg actacttcac gtccaccgag
1621 cccagtagt ag

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