

Certificate of Analysis

Src, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-326, 14-326-K, 14-326M

Parent Lot # WAB0003

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, full-length, human Src expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA-agarose. Purity 95.3% by SDS-PAGE and Coomassie blue staining. MW = 61.7kDa.

Specific Activity (Parent lot# WAB0003): 1022U/mg, where one unit of Src, active activity is defined as 1nmol phosphate incorporated into 250µM cdc2 substrate peptide (cat# 12-140) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 1.128mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 5% glycerol, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

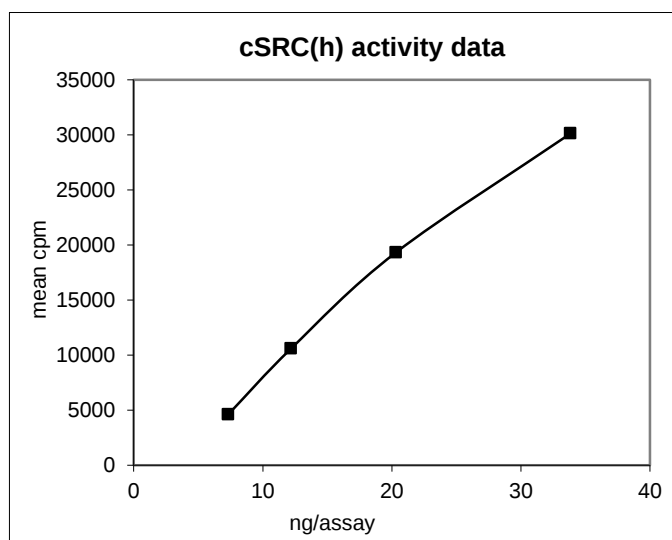
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

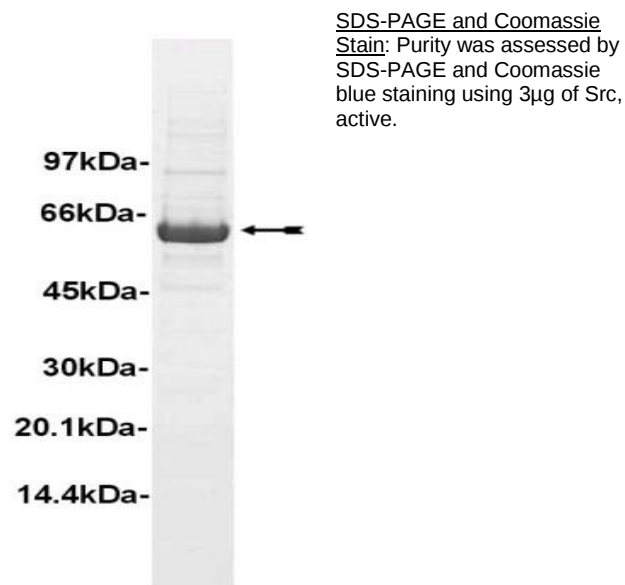
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 7–34ng of this lot of enzyme phosphorylated 250µM cdc2 substrate peptide 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 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. **cdc2 peptide (KVEKIGEGTYGVVYK):** Use at a final assay concentration of 250 μ M. Prepare a 2.5mM stock and add 2.5 μ l of stock per assay point.
3. **Src, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 7–34ng 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 appropriate wells.
2. Add 2.5 μ l of **cdc2 substrate peptide**.
3. Add **2.5 μ l (7–34ng) Src, 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 Sequence Information

Protein	human Src
Tags	N-Terminal 6His
Native sequence	M14 of the recombinant protein is equivalent to M1 of human Src
Accession number	EMBL K03218

Recombinant Src amino acid sequence:

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1 MSYYHHHHH DEFMSNKS PKDASQRRS LEPAENVHGA GGAFFPASQT PSKPASADGH
61 RGPAAAFAPA AAEPKLFGG NSSDTVTSPQ RAGPLAGGVT TFVALYDYES RTETDLSEFK
121 GERLQIVNNT EGDWWLAHSL STGQTGYIPS NYVAPSDSIQ AEEWYFGKIT RRESERLLL
181 AENPRGTFLV RESETTKGAY CLSVSDFDNA KGLNVKHYKI RKLDSGGFYI TSRTQFNSLQ
241 QLVAYYSKHA DGLCHRLTTV CPTSKPQTQG LAKDAWEIPR ESLRLEVKLQ QGCFGEVWMG
301 TWNGTTRVAI KTLKPGTMSP EAFLQEAQVM KKLREHELVQ LYAVVSEEPY YIVTEYMSKG
361 SLLDFLKGET GKYLRLPQLV DMAAQIASGM AYVERMNYVH RDLRAANILV GENLVCKVAD
421 FGLARLIEDN EYTARQGAKE PIKWTAPEAA LYGRFTIKSD VWSFGILLTE LTTKGRVPYP
481 GMVNREVLQD VERGYRMPCP PECPESLHDL MCQCWRKEPE ERPTFEYLQA FLEDYFTSTE
541 PQYQPGENL

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Recombinant Src nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gatgaattca tgggtagcaa caagagcaag
61 cccaaggatg ccagccagcg ggcgcgagc ctggagcccg ccgagaacgt gcacggcgct
121 ggcgggggag cttccccgc ctcgcagacc cccagcaagc cagcctcggc cgacggccac
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1621 cccagttacc agcccgggga gaacctctag

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