

Certificate of Analysis

Src (T341M), active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-748, 14-748-K, 14-748M

Parent Lot # 1635816

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, containing the T341M mutation, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA-agarose.

This T341M mutation is targeted at the 'gatekeeper' residue in the ATP-binding pocket of the catalytic domain. This mutation has been demonstrated to affect sensitivity to selective inhibitors (Blencke et al., (2004), Chem. Biol., 11, 691-701).

Purity 60.8% by SDS-PAGE and Coomassie blue staining. MW = 61.7kDa

Specific Activity (Parent lot# 1635816): 901U/mg, where one unit of Src (T341M), active activity is defined as 1nmol phosphate incorporated into 500µM (GGEEEEYFELVKKKK) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.732mg/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.

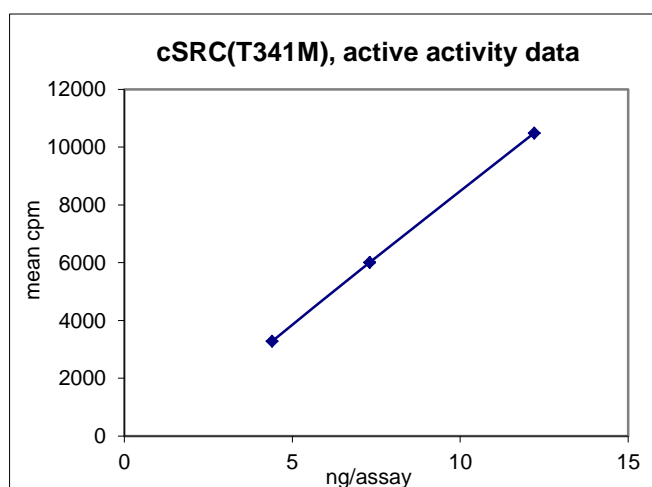
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

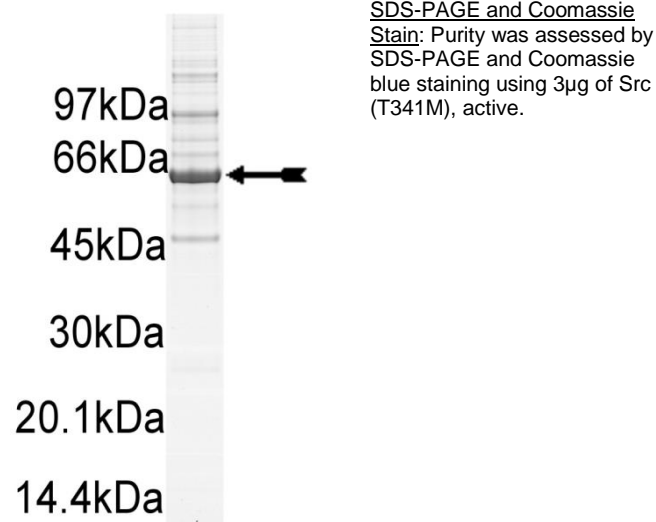
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 4.4–12.2ng of this lot of enzyme phosphorylated 500µM (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 (T341M) 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. **(GGESEEFELVKKKK):** 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. **NaCl:** Use at a final assay concentration of 100mM. Make a 3M stock. Add 0.83 μ l of stock per assay point.
4. **Src (T341M), active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 4.4–12.2ng per assay point.
5. **[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 (GGESEEFELVKKKK).
3. Add **2.5 μ l (4.4–12.2ng) Src (T341M), active.**
4. Add 0.83 μ l of 3M NaCl.
5. Add 4.17 μ l of dH₂O.
6. Add 10 μ l of diluted [γ -³³P]ATP mixture.
7. Incubate for 10 minutes at 30°C.
8. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
9. Transfer a 10 μ l aliquot onto the appropriate area of a **P30 Filtermat.**
10. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
11. Wash the filtermat once for 2 minutes with methanol.
12. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
13. 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 (T341M) Sequence Information

<u>Protein</u>	human Src (T341M)
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M14 of recombinant sequence is equivalent to M1 of native human Src
<u>Accession number</u>	EMBL K03218

Recombinant Src (T341M) amino acid sequence:

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1 MSYYHHHHH DEFMGSNKS PKDASQRRRS LEPAENVHGA GGGAFPASQT PSKPASADGH
61 RGPAAAFAPA AAEPKLFGGF NSSDVTSPQ RAGPLAGGVT TFVALYDYES RTETDLSEFKK
121 GERLQIVNNT EGDWLAHSL STGQTGYIPS NYVAPSDSIQ AEEWYFGKIT RRESERLLL
181 AENPRGTFVL RESETTKGAY CLSVSDFDNA KGLNVKHYKI RKLDSGGFYI TSRTQFNSLQ
241 QLVAYYSKHA DGLCHRLTTV CPTSKPQTQG LAKDAWEIPR ESLRLEVKLG QGCFGEVWMG
301 TWNGTTRVAI KTLKPGTMSP EAFLQEAQVM KCLRHEKLVQ LYAVVSEEPI YIVMEYMSKG
361 SLLDFLKGET GKYLRLPQLV DMAAQIASGM AYVERMNYVH RDLRAANILV GENLVCKVAD
421 FGLARLIEDN EYTARQGAKF PIKWTAPEAA LYGRFTIKSD VWSFGILLTE LTTKGRVPYP
481 GMVNREVLQD VERGYRMPCP PECPELHDL MCQCWRKEPE ERPTFEYLQA FLEDYFTSTE
541 PQYQPGENL
  
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Recombinant Src (T341M) nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gatgaattca tgggtagcaa caagagcaag
61 cccaaggatg ccagccagcg gcgccgagc ctggagcccg ccgagaacgt gcacggcgct
121 ggcggggcg cttccccgc ctgcagacc ccagcaagc cagcctcggc cgacggccac
181 gcggcccca gcgggcctt cgccccgcg gccgcccagc ccaagctgtt cggaggcttc
241 aactcctcgg acaccgtcac ctccccgag agggcgggccc cgctggccgg tggagtgacc
301 acctttgtgg ccctctatga ctatgagtct aggacggaga cagacctgtc cttcaagaaa
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481 gctgaggagt ggtatthttg caagatcacc agacgggagt cagagcggtt actgctcaat
541 gcagagaacc cgagagggac cttcctcgtg cgagaaagtg agaccacgaa aggtgcctac
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1141 gacatggctg ctcatcgc ctcatcagc gcgtacgtgg agcggatgaa ctacgtccac
1201 cgggaccttc gcgcagcaa catcctggtg ggagagaacc tgggtgtgaa agtggccgac
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1321 cccatcaagt ggacggctcc agaagctgcc ctctatggcc gcttcacat caagtccgac
1381 gtgtggtcct tcgggaccc tctgactgag ctaccacaa agggacgggt gccctaccct
1441 gggatggtga accgcgaggt gctggaccag gtggagcggg gctaccggat gccctgcccc
  
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1501 ccggagtgtc ccgagtcct gcacgacctc atgtgccagt gctggcggaa ggagcctgag
1561 gagcggccca cttcgagta cctgcaagcc ttcctggagg actacttcac gtccaccgag
1621 ccccagtacc agcccgggga gaacctctag
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