

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active Src [1 - 536]

<u>Enzyme description:-</u>	Src [1 - 536]
<u>Clone number:-</u>	DU 19041
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal His(6)
<u>Purification method:-</u>	Ni ²⁺ -NTA agarose

Calculated molecular mass:-

Monoisotopic 63, 232.45 daltons
Average Mass 63, 272.49 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.46

Purity:- >85 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70 °C

Assay buffer:-

50 mM Tris-HCl pH 7.5, 0.1 % 2-mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc

Substrate:-

KVEKIGEGTYGVVYK [Residues 5 – 20 of human CDK2]
Final concentration: 250 µM

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Clone Data Sheet

Src [1 - 536]

<u>Protein</u>	Src [1 - 536]
<u>Clone number</u>	DU 19041
<u>Species</u>	Human
<u>Accession no</u>	NM_005417.3
<u>Tags</u>	N-terminal His(6)
<u>Baculovirus expressed protein</u>	MSYYHHHHHHDIPTTENLYFQGAMDPEFMGSNKS KPKDASQRRRSLEPAEN VHGAGGGAF PASQTPSKPASADGHRGPSAAFAPAAAEPKLFGGFNSSDVT SPQRAGPLAGGVTTFVALYDYESRTETDLSFKKGERLQIVNNTEGDWL SLSTGQTGYIPSNYVAPSDSIQAEWYFGKITRRESERLLLNAENPRGTFL VRESETTKGAYCLSVSDFDNAKGLNVKHYKIRKLDSGGFYITSRTQFN SLQQLVAYYSKHADGLCHRLTTVCPTSKPQTQGLAKDAWEIPRESLRLE VKLGQ GCFGEVWMGTWNGTTRVAIKTLKPGTMSPEAFLEAQVMKKLR HEKLVQLY AVVSEEP IYIVTEYMSKGSLLDFLKGETGKYLRLPQLVD MAAQIASGMAYV ERMNYVHRDLRAANILVGENLVCKVADFGLARLIE DNEYTARQGA KFP I KWTAPEAALYGRFTIKSDVWSFGILLTELTT KGRVPYPGMVNREVL DQVERGYRMPCPPECPESLHDL MCQCWRKE PEERPTFEY LQAFLEDYFTSTEPQYQPG ENL
<u>Native sequence</u>	Amino acids M1 – L536 (end) of human Src. Residue M29 of the fusion protein is equivalent to M2 of human Src. The His(6) tag is located at residues 5 – 10.
<u>Protease cleavage</u>	rTEV (<u>ENLYFOG</u>) residues 18 - 24
<u>Cloning sites</u>	<i>Eco</i> R1 and <i>Xho</i> I sites of pFastBAC HTa
<u>Nucleotide sequence of insert</u>	gaattcATGGGTAGCAACAAGAGCAAGCCCAAGGATGCCAGCCAGCGGCGC CGCAGCCTGGAGCCCGCCGAGAACGTGCACGGCGCTGGCGGGGGCGCTTTC

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CCCAGCGCGGCCTTCGCCCCGCGGCCGCCGAGCCCAAGCTGTTCGGAGGC
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