Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active Src [1 - 536]

Enzyme description:- Src [1 - 536]

Clone number:- DU 19041

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal His(6)

Purification method:- Ni^{2+}-NTA agarose

Expression level:- 5 mg/L

Calculated molecular mass:-
Monoisotopic 63, 232.45daltons
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:- Standard filter binding assay

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

Specific activity range:- To be fully determined

Clone Data Sheet

Src [1 - 536]

Protein	Src [1 - 536]
Clone number	DU 19041
Species	Human
Accession no	NM_005417.3
Tags	N-terminal His(6)
Baculovirus expressed protein	MSYYHHHHHHDIPTTENLYFQGAMDPEF**MGSNKSKPKDASQRRRSLEPAEN** **VHGAGGGAFPASQTPSKPASADGHRGPSAAFAPAAAEPKLFGGFNSSDTVT** **SPQRAGPLAGGVTTFVALYDYESRTETDLSFKKGERLQIVNNTEGDWWLAH** **SLSTGQTGYIPSNYVAPSDSIQAEEWYFGKITRRESERLLLNAENPRGTFL** **VRESETTKGAYCLSVSDFDNAKGLNVKHYKIRKLDSGGFYITSRTQFNSLQ** **QLVAYYSKHADGLCHRLTTVCPTSKPQTQGLAKDAWEIPRESLRLEVKLGQ** **GCFGEVWMGTWNGTTRVAIKTLKPGTMSPEAFLQEAQVMKKLRHEKLVQLY** **AVVSEEPIYIVTEYMSKGSLLDFLKGETGKYLRLPQLVDMAAQIASGMAYV** **ERMNYVHRDLRAANILVGENLVCKVADFGLARLIEDNEYTARQGAKFPIKW** **TAPEAALYGRFTIKSDVWSFGILLTELTTKGRVPYPGMVNREVLDQVERGY** **RMPCPPECPESLHDLMCQCWRKEPEERPTFEYLQAFLEDYFTSTEPQYQPG** **ENL**
Native sequence	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.
Protease cleavage	rTEV (ENLYFQG) residues 18 - 24
Cloning sites	*Eco*R1 and *Xho*1 sites of pFastBAC HTa

Nucleotide sequence of insert

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gaattcATGGGTAGCAACAAGAGCAAGCCCAAGGATGCCAGCCAGCGGCGC
CGCAGCCTGGAGCCCGCCGAGAACGTGCACGGCGCTGGCGGGGGCGCTTTC
CCCGCCTCGCAGACCCCCAGCAAGCCAGCCTCGGCCGACGGCCACCGCGGC
CCCAGCGCGGCCTTCGCCCCCGCGGCCGCCGAGCCCAAGCTGTTCGGAGGC
TTCAACTCCTCGGACACCGTCACCTCCCCGCAGAGGGCGGGCCCGCTGGCC
GGTGGAGTGACCACCTTTGTGGCCCTCTATGACTATGAGTCTAGGACGGAG
ACAGACCTGTCCTTCAAGAAAGGCGAGCGGCTCCAGATTGTCAACAACACA
GAGGGAGACTGGTGGCTGGCCCACTCGCTCAGCACAGGACAGACAGGCTAC
ATCCCCAGCAACTACGTGGCGCCCTCCGACTCCATCCAGGCTGAGGAGTGG
TATTTTGGCAAGATCACCAGACGGGAGTCAGAGCGGTTACTGCTCAATGCA
GAGAACCCGAGAGGGACCTTCCTCGTGCGAGAAAGTGAGACCACGAAAGGT
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CGCACCCAGTTCAACAGCCTGCAGCAGCTGGTGGCCTACTACTCCAAACAC
GCCGATGGCCTGTGCCACCGCCTCACCACCGTGTGCCCCACGTCCAAGCCG
CAGACTCAGGGCCTGGCCAAGGATGCCTGGGAGATCCCTCGGGAGTCGCTG
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CTCACCACAAAGGGACGGGTGCCCTACCCTGGGATGGTGAACCGCGAGGTG
CTGGACCAGGTGGAGCGGGGCTACCGGATGCCCTGCCCGCCGGAGTGTCCC
GAGTCCCTGCACGACCTCATGTGCCAGTGCTGGCGGAAGGAGCCTGAGGAG
CGGCCCACCTTCGAGTACCTGCAGGCCTTCCTGGAGGACTACTTCACGTCC
ACCGAGCCCCAGTACCAGCCCGGGGAGAACCTCtagctcgag
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