MRC PPU Reagents and Services

Standard Operating Procedure

Preparation of active SRPK1 [2 – 654]

Enzyme description:- SRPK1 [2 - 654]

Clone number:- DU 967

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 100, 854.88 daltons
Average Mass 100, 918.62 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.79

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:-
RSRSRSRSRSRSRSR residues 204 – 218 of human ASF-1/SF-2
Final concentration: 300 μM

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Clone Data Sheet

SRPK1 [2 - 654]

Protein	SRPK1 [2 – 654]
Clone number	DU 967
Species	Human
Accession number	NM_003137
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**ERKVLALQA** **RKKRTKAKKDKAQRKSETQHRGSAPHSESDLPEQEEEILGSDDDEQED** **PNDYCKGGYHLVKIGDLFNGRYHVIRKLGWGHFSTVWLSWDIQGKKFV** **AMKVVKSAEHYTETALDEIRLLKSVRNSDPNDPNREMVVQLLDDFKIS** **GVNGTHICMVFEVLGHHLLKWIIKSNYQGLPLPCVKKIIQQVLQGLDY** **LHTKCRIIHTDIKPENILLSVNEQYIRRLAAEATEWQRSGAPPPSGSA** **VSTAPQPKPADKMSKNKKKKLKKKQKRQAELLEKRMQEIEEMEKESGP** **GQKRPNKQEESESPVERPLKENPPNKMTQEKLEESSTIGQDQTLMERD** **TEGGAAEINCNGVIEVINYTQNSNNETLRHKEDLHNANDCDVQNLNQE** **SSFLSSQNGDSSTSQETDSCTPITSEVSDTMVCQSSSTVGQSFSEQHI** **SQLQESIRAEIPCEDEQEQEHNGPLDNKGKSTAGNFLVNPLEPKNAEK** **LKVKIADLGNACWHKHFTEDIQTRQYRSLEVLIGSGYNTPADIWSTAC** **MAFELATGDYLFEPHSGEEYTRDEDHIALIIELLGKVPRKLIVAGKYS** **KEFFTKKGDLKHITKLKPWGLFEVLVEKYEWSQEEAAGFTDFLLPMLE** **LIPEKRATAAECLRHPWLNS**
Native sequence	Amino acids E2 – S654 (end) of human SRPK1. Residue E2 of the fusion protein is equilivalent to E232 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	PreScission (LEVLFQGPL) at residues 221 – 229
Cloning sites	*Bam*H1 and *Eco*R1 site of pGEX6P-1

Nucleotide sequence of insert

GGATCCGAGCGGAAAGTGCTTGCGCTCCAGGCCCGAAAGAAAAGGACC
AAGGCCAAGAAGGACAAAGCCCAAAGGAAATCTGAAACTCAGCACCGA
GGCTCTGCTCCCCACTCTGAGAGTGATCTACCAGAGCAGGAAGAGGAG
ATTCTGGGATCTGATGATGATGAGCAAGAAGATCCTAATGATTATTGT
AAAGGAGGTTATCATCTTGTGAAAATTGGAGATCTATTCAATGGGAGA
TACCATGTGATCCGAAAGTTAGGCTGGGGACACTTTTCAACAGTATGG
TTATCATGGGATATTCAGGGGAAGAAATTTGTGGCAATGAAAGTAGTT
AAAAGTGCTGAACATTACACTGAAACAGCACTAGATGAAATCCGGTTG
CTGAAGTCAGTTCGCAATTCAGACCCTAATGATCCAAATAGAGAAATG
GTTGTTCAACTACTAGATGACTTTAAAATATCAGGAGTTAATGGAACA
CATATCTGCATGGTATTTGAAGTTTTGGGGCATCATCTGCTCAAGTGG
ATCATCAAATCCAATTATCAGGGGCTTCCACTGCCTTGTGTCAAAAAA
ATTATTCAGCAAGTGTTACAGGGTCTTGATTATTTACATACCAAGTGC
CGTATCATCCACACTGACATTAAACCAGAGAACATCTTATTGTCAGTG
AATGAGCAGTACATTCGGAGGCTGGCTGCAGAAGCAACAGAATGGCAG
CGATCTGGAGCTCCTCCGCCTTCCGGATCTGCAGTCAGTACTGCTCCC
CAGCCTAAACCAGCTGACAAAATGTCAAAGAATAAGAAGAAGAAATTG
AAGAAGAAGCAGAAGCGCCAGGCAGAATTACTAGAGAAGCGAATGCAG
GAAATTGAGGAAATGGAGAAAGAGTCGGGCCCTGGGCAAAAAAGACCA
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AACAGTAATAATGAAACATTGAGACATAAAGAGGATCTACATAATGCT
AATGACTGTGATGTCCAAAATTTGAATCAGGAATCTAGTTTCCTAAGC
TCCCAAAATGGAGACAGCAGCACATCTCAAGAAACAGACTCTTGTACA
CCTATAACATCTGAGGTGTCAGACACCATGGTGTGCCAGTCTTCCTCA
ACTGTAGGTCAGTCATTCAGTGAACAACACATTAGCCAACTTCAAGAA
AGCATTCGGGCAGAGATACCCTGTGAAGATGAACAAGAGCAAGAACAT
AACGGACCACTGGACAACAAAGGAAAATCCACGGCTGGAAATTTTCTT
GTTAATCCCCTTGAGCCAAAAAATGCAGAAAAGCTCAAGGTGAAGATT
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CAAACAAGGCAATATCGTTCCTTGGAAGTTCTAATCGGATCTGGCTAT
AATACCCCTGCTGACATTTGGAGCACGGCATGCATGGCCTTTGAACTG
GCCACAGGTGACTATTTGTTTGAACCTCATTCAGGGGAAGAGTACACT
CGAGATGAAGATCACATTGCATTGATCATAGAACTTCTGGGGAAGGTG
CCTCGCAAGCTCATTGTGGCAGGAAAATATTCCAAGGAATTTTTCACC
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TTTGAGGTTCTAGTGGAGAAGTATGAGTGGTCGCAGGAAGAGGCAGCT
GGCTTCACAGATTTCTTACTGCCCATGTTGGAGCTGATCCCTGAGAAG
AGAGCCACTGCCGCCGAGTGTCTCCGGCACCCTTGGCTTAACTCCtaa
gaattc