MRC PPU Reagents and Services

Standard Operating Procedure

Preparation of SRPK1 D497A [2 – 655]

Enzyme description:- SRPK1 D497A [2 - 655]

Clone number:- DU 66208

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 100, 909.96 daltons
Average Mass 100, 973.74 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.83

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 D497A [2 - 655]

Protein	SRPK1 D497A [2 – 655]
Clone number	DU 66208
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** **LKVKIAALGNACWVHKHFTEDIQTRQYRSLEVLIGSGYNTPADIWSTA** **CMAFELATGDYLFEPHSGEEYTRDEDHIALIIELLGKVPRKLIVAGKY** **SKEFFTKKGDLKHITKLKPWGLFEVLVEKYEWSQEEAAGFTDFLLPML** **ELIPEKRATAAECLRHPWLNS**
Native sequence	Amino acids E2 – S655 (end) of human SRPK1. Residue E2 of the fusion protein is equivalent to E232 of the native enzyme. The GST tag is located at residues 1 – 220. The enzyme has a D497A mutation. Residue D497A is equivalent to A727 of the fusion protein.
Protease cleavage	PreScission (LEVLFQGP) at residues 221 – 228
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
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CAGCCTAAACCAGCTGACAAAATGTCAAAGAATAAGAAGAAGAAATTG
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AACCCACCTAATAAAATGACCCAAGAAAAACTTGAAGAGTCAAGTACC
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GCAGAAATTAATTGCAATGGAGTGATTGAAGTCATTAATTATACTCAG
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CCTATAACATCTGAGGTGTCAGACACCATGGTGTGCCAGTCTTCCTCA
ACTGTAGGTCAGTCATTCAGTGAACAACACATTAGCCAACTTCAAGAA
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TATAATACCCCTGCTGACATTTGGAGCACGGCATGCATGGCCTTTGAA
CTGGCCACAGGTGACTATTTGTTTGAACCTCATTCAGGGGAAGAGTAC
ACTCGAGATGAAGATCACATTGCATTGATCATAGAACTTCTGGGGAAG
GTGCCTCGCAAGCTCATTGTGGCAGGAAAATATTCCAAGGAATTTTTC
ACCAAAAAAGGTGACCTGAAACATATCACGAAGCTGAAACCTTGGGGC
CTTTTTGAGGTTCTAGTGGAGAAGTATGAGTGGTCTCAGGAAGAGGCA
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AAGAGAGCCACTGCCGCCGAGTGTCTCCGGCACCCTTGGCTtaactcc
taagaattc