MRC PPU Reagents and Services

Standard Operating Procedure

Preparation of active SRPK2 [1 - 699]

Enzyme description:- SRPK2 [1 - 699]

Clone number:- DU 36135

Source:- Recombinant

Expression system:- *E.coli*,

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 105, 785.92 daltons
Average Mass 105, 852.91 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.21

Purity:- >80 %

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM sucrose, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.03 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

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

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Clone Data Sheet

SRPK2 [1 - 699]

Protein	SRPK2 [1 - 699]
Clone number	DU 35135
Species	Human
Accession number	NM_182692.1
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MSSRKVLAI QARKRRPKREKHPKKPEPQQKAPLVPPPPPPPPPPPPLPDPTPPEPE EEILGSDDEEQEDPADYCKGGYHPVKIGDLFNGRYHVIRKLGWGHFST VWLCWDMQGKRFVAMKVVKSAQHYTETALDEIKLLKCVRESDPSDPNK DMVVQLIDDFKISGMNGIHVCMVFEVLGHHLLKWIIKSNYQGLPVRCV KSIIRQVLQGLDYLHSKCKIIHTDIKPENILMCVDDAYVRRMAAEATE WQKAGAPPPSGSAVSTAPQQKPIGKISKNKKKKLKKKQKRQAELLEKR LQEIEELEREAERKIIEENITSAAPSNDQDGEYCPEVKLKTTGLEEAA EAETAKDNGEAEDQEEKEDAEKENIEKDEDDVDQELANIDPTWIESPK TNGHIENGPFSLEQQLDDEDDDEEDCPNPEEYNLDEPNAESDYTYSSS YEQFNGELPNGRHKIPESQFPEFSTSLFSGSLEPVACGSVLSEGSPLT EQEESSPSHDRSRTVSASSTGDLPKAKTRAADLLVNPLDPRNADKIRV KIADLGNACWVHKHFTEDIQTRQYRSIEVLIGAGYSTPADIWSTACMA FELATGDYLFEPHSGEDYSRDEDHIAHIIELLGSIPRHFALSGKYSRE FFNRRGELRHITKLKPWSLFDVLVEKYGWPHEDAAQFTDFLIPMLEMV PEKRASAGECLRHPWLNS**

Native sequence	Amino acids M1 – S699 (end) of human SRPK2. Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	Prescission site (LEVLFQGP) at residues 221 - 228
Cloning sites	*Bg*III and *Not*1 into *Bam*H1 and *Not*1 sites of pGEX-6P-3

Nucleotide sequence of insert

agatctATGAGCTCCCGGAAAGTGCTGGCCATTCAGGCCCGAAAGCGG
AGGCCGAAAAGAGAGAAACATCCGAAAAAGCCGGAGCCTCAACAGAAA
GCTCCTTTAGTTCCTCCTCCTCCACCGCCACCACCACCACCACCGCCA
CCTTTGCCAGACCCCACACCCCCGGAGCCAGAGGAGGAGATCCTGGGA
TCAGATGATGAGGAGCAAGAGGACCCTGCGGACTACTGCAAAGGTGGA
TATCATCCAGTGAAAATTGGAGACCTCTTCAATGGCCGGTATCATGTT
ATTAGAAAGCTTGGATGGGGGCACTTCTCTACTGTCTGGCTGTGCTGG
GATATGCAGGGGAAAAGATTTGTTGCAATGAAAGTTGTAAAAAGTGCC
CAGCATTATACGGAGACAGCCTTGGATGAAATAAAATTGCTCAAATGT
GTTCGAGAAAGTGATCCCAGTGACCCAAACAAAGACATGGTGGTCCAG
CTCATTGACGACTTCAAGATTTCAGGCATGAATGGGATACATGTCTGC
ATGGTCTTCGAAGTACTTGGCCACCATCTCCTCAAGTGGATCATCAAA
TCCAACTATCAAGGCCTCCCAGTACGTTGTGTGAAGAGTATCATTCGA
CAGGTCCTTCAAGGGTTAGATTACTTACACAGTAAGTGCAAGATCATT
CATACTGACATAAAGCCGGAAAATATCTTGATGTGTGTGGATGATGCA
TATGTGAGAAGAATGGCAGCTGAGGCCACTGAGTGGCAGAAAGCAGGT
GCTCCTCCTCCTTCAGGGTCTGCAGTGAGTACGGCTCCACAGCAGAAA
CCTATAGGAAAAATATCTAAAAACAAAAAGAAAAAACTGAAAAAGAAA
CAGAAGAGGCAGGCTGAGTTATTGGAGAAGCGCCTGCAGGAGATAGAA
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AAAGAAAACATTGAAAAAGATGAAGATGATGTAGATCAGGAACTTGCG
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GAGAATGGCCCATTCTCACTGGAGCAGCAACTGGACGATGAAGATGAT
GATGAAGAAGACTGCCCAAATCCTGAGGAATATAATCTTGATGAGCCA
AATGCAGAAAGTGATTACACATATAGCAGCTCCTATGAACAATTCAAT
GGTGAATTGCCAAATGGACGACATAAAATTCCCGAGTCACAGTTCCCA
GAGTTTTCCACCTCGTTGTTCTCTGGATCCTTAGAACCTGTGGCCTGC
GGCTCTGTGCTTTCTGAGGGATCACCACTTACTGAGCAAGAGGAGAGC
AGTCCATCCCATGACAGAAGCAGAACGGTTTCAGCCTCCAGTACTGGG
GATTTGCCAAAAGCAAAAACCCGGGCAGCTGACTTGTTGGTGAATCCC
CTGGATCCGCGGAATGCAGATAAAATTAGAGTAAAAATTGCTGACCTG
GGAAATGCTTGTTGGGTGCATAAACACTTCACGGAAGACATCCAGACG
CGTCAGTACCGCTCCATAGAGGTTTTAATAGGAGCGGGGTACAGCACC
CCTGCGGACATCTGGAGCACGGCGTGTATGGCATTTGAGCTGGCAACG
GGAGATTATTTGTTTGAACCACATTCTGGGGAAGACTATTCCAGAGAC
GAAGACCACATAGCCCACATCATAGAGCTGCTAGGCAGTATTCCAAGG
CACTTTGCTCTATCTGGAAAATATTCTCGGGAATTCTTCAATCGCAGA
GGAGAACTGCGACACATCACCAAGCTGAAGCCCTGGAGCCTCTTTGAT
GTACTTGTGGAAAAGTATGGCTGGCCCCATGAAGATGCTGCACAGTTT
ACAGATTTCCTGATCCCGATGTTAGAAATGGTTCCAGAAAAACGAGCC
TCAGCTGGCGAATGCCTTCGGCATCCTTGGTTGAATTCTtagcggccg
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