MRC PPU REAGENTS

Standard Operating Procedure

Preparation of SRPK3 [1 – 567]

Enzyme description:- SRPK3 [1 - 567]

Clone number:- DU 27340

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 88, 781.82 daltons
Average Mass 88, 838.35 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.43

Enzyme storage buffer:-

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

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

MRC PPU REAGENTS

Clone Data Sheet

SRPK3 [1 - 567]

Protein SRPK3 [1 - 567]

Clone number DU 27340

Species Human

Accession number NM_014370.3

Tags N-terminal GST

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG
LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA
VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS
KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MSASTGGGGDSGGS**
GGSSSSSQASCGPESSGSELALATPVPQMLQGLLGSDDEEQEDPKDYCK
GGYHPVKIGDVFNGRYHVVRKLGWGHFSTVWLCWDIQRKRFVALKVVKS
AGHYTETAVDEIKLLKCVRDSDPSDPKRETIVQLIDDFRISGVNGVHVC
MVLEVLGHQLLKWIIKSNYQGLPVPCVKSIVRQVLHGLDYLHTKCKIIH
TDIKPENILLCVGDAYIRRLAAEATEWQQAGAPPPSRSIVSTAPQEVLQ
TGKLSKNKRKKMRRKRKQQKRLLEERLRDLQRLEAMEAATQAEDSGLRL
DGGSGSTSSSGCHPGGARAGPSPASSSPAPGGGRSLSAGSQTSGFSGSL
FSPASCSILSGSSNQRETGGLLSPSTPFGASNLLVNPLEPQNADKIKIK
IADLGNACWVHKHFTEDIQTRQYRAVEVLIGAEYGPPADIWSTACMAFE
LATGDYLFEPHSGEDYSRDEDHIAHIVELLGDIPPAFALSGRYSREFFN
RRGELRHIHNLKHWGLYEVLMEKYEWPLEQATQFSAFLLPMMEYIPEKR
ASAADCLQHPWLNP

Native sequence Amino acids M1 – P567 (end) of human SRPK3.
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 (LEVLFQGP) residues 221 – 228.

Cloning sites *Bam*H1 and *Not*1 sites of pGex6P1

Nucleotide Sequence Of Insert

ggatccATGAGCGCCAGCACGGGCGGTGGTGGGGACAGCGGCGGCAGCGGCGGCAGTAGCA
GCAGCTCACAGGCCTCCTGCGGGCCCGAGTCCTCGGGCTCCGAACTAGCCCTGGCCACACC
GGTGCCTCAGATGCTGCAGGGCCTTCTGGGCTCCGACGACGAGGAACAGGAAGACCCCAAA
GACTACTGCAAGGGCGGCTACCACCCTGTGAAGATCGGCGACGTGTTCAATGGGCGGTACC
ACGTGGTGCGCAAACTGGGCTGGGGCCACTTCTCCACCGTCTGGCTCTGCTGGGACATCCA
GCGCAAGCGCTTTGTGGCCCTCAAAGTGGTGAAGAGTGCGGGGCATTACACGGAGACAGCT
GTGGATGAGATCAAGCTCCTGAAATGTGTCCGGGACAGCGACCCCAGTGACCCCAAAAGAG
AGACCATTGTCCAGCTCATTGATGACTTCAGGATCTCAGGAGTCAATGGAGTCCATGTGTG
CATGGTGCTGGAGGTGCTGGGCCACCAGCTCCTCAAATGGATCATCAAGTCCAACTACCAG
GGCCTGCCCGTGCCCTGCGTGAAGAGCATCGTGAGGCAGGTGCTGCACGGCCTGGACTACC
TCCACACCAAGTGCAAGATCATCCACACGGACATCAAGCCCGAGAACATCTTGCTGTGTGT
GGGGGACGCTTACATCAGGCGCCTGGCTGCCGAGGCCACGGAGTGGCAACAGGCAGGGGCG
CCGCCCCCCTCCCGCTCCATAGTCAGCACTGCCCCCCAGGAGGTCTTGCAGACCGGTAAGC
TGTCCAAAAACAAGAGGAAGAAGATGAGGCGCAAACGGAAACAGCAGAAGCGGCTGCTGGA
GGAGCGGCTGCGGGACCTGCAGAGGCTGGAGGCCATGGAGGCTGCCACCCAGGCTGAGGAC
TCTGGCTTGAGACTAGACGGGGGCAGCGGCTCCACATCCTCTTCAGGCTGTCACCCCGGGG
GCGCCAGAGCAGGTCCCTCCCCAGCCTCTTCCTCCCCCGCCCCGGGGGGCGGCCGTAGCCT
CAGCGCGGGCTCACAGACCTCAGGCTTCTCCGGCTCCCTCTTCTCTCCTGCCTCCTGCTCC
ATCCTCTCCGGCTCGTCCAATCAGCGAGAGACCGGGGGCCTCCTGTCGCCTAGCACACCAT
TCGGTGCCTCGAACCTCCTGGTGAACCCCCTGGAGCCCCAAAATGCAGATAAGATCAAGAT
CAAGATCGCAGACCTGGGCAACGCCTGCTGGGTGCACAAGCACTTCACGGAAGACATCCAG
ACTCGGCAGTACCGGGCCGTCGAGGTGCTGATCGGCGCCGAATACGGCCCCCCGGCAGACA
TCTGGAGCACAGCCTGCATGGCCTTCGAGCTGGCCACTGGTGACTACCTGTTCGAGCCGCA
TTCTGGAGAAGACTACAGTCGTGATGAGGACCACATCGCTCACATAGTGGAGCTTCTGGGG
GACATCCCCCCAGCCTTCGCCCTCTCAGGCCGCTATTCCCGGGAGTTCTTCAACCGGAGAG
GAGAGCTGCGGCACATCCACAATCTCAAGCACTGGGGCCTGTACGAGGTACTCATGGAAAA
GTACGAGTGGCCCCTAGAGCAGGCCACACAGTTCAGCGCCTTTCTGCTGCCCATGATGGAG
TACATCCCCGAAAAGCGGGCCAGTGCCGCTGACTGCCTCCAGCACCCCTGGCTCAACCCCt
agcggccgca