MRC PPU Reagents and Services

Standard Operating Procedure

Preparation of active MRCK alpha [1 - 473]

Enzyme description:- MRCK alpha [1 – 473]

Clone number:- DU 62948

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 81, 157.53 daltons
Average Mass 81, 209.98 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.25

Purity:- >80 %

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 deg C

Assay buffer:-
50 mM Tris-HCl pH 7.5, 10 mM DTT, 10 mM magnesium acetate, 0. 1mM EGTA

Substrate:-
KKRNRTLTV Final concentration: 300 uM

MRC PPU Reagents and Services

Clone Data Sheet

MRCK alpha [1 – 473]

Protein	MRCK alpha [1 – 473]
Clone number	DU 62948
Species	Human
Accession number	NM_003607.3
Tags	N-terminal GST

Baculovirus expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG
LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA
VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS
KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MSGEVRLRQLEQFI**
LDGPAQTNGQCFSVETLLDILICLYDECNNSPLRREKNILEYLEWAKPF
TSKVKQMRLHREDFEILKVIGRGAFGEVAVVKLKNADKVFAMKILNKWE
MLKRAETACFREERDVLVNGDNKWITTLHYAFQDDNNLYLVMDYYVGGD
LLTLLSKFEDRLPEDMARFYLAEMVIAIDSVHQLHYVHRDIKPDNILMD
MNGHIRLADFGSCLKLMEDGTVQSSVAVGTPDYISPEILQAMEDGKGRY
GPECDWWSLGVCMYEMLYGETPFYAESLVETYGKIMNHKERFQFPAQVT
DVSENAKDLIRRLICSREHRLGQNGIEDFKKHPFFSGIDWDNIRNCEAP
YIPEVSSPTDTSNFDVDDDCLKNSETMPPPTHTAFSGHHLPFVGFTYTS
SCVLSDRSCLRVTAGPTSLDLDVNVQRTLDNNLATEAYERRIKRLEQEK
LELSRKLQESTQTVQALQ

Native sequence	Amino acids M1 – Q473 (end residue is P1719) of human MRCK alpha. 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 pFastBac-GST

Nucleotide sequence of insert

gtcgacATGGATACAAAATCTATTCTAGAAGAACTTCTTCTCAAAAGA
TCACAGCAAAAGAAGAAAATGTCACCAAATAATTACAAAGAACGGCTT
TTTGTTTTGACCAAAACAAACCTTTCCTACTATGAATATGACAAAATG
AAAAGGGGCAGCAGAAAAGGATCCATTGAAATTAAGAAAATCAGATGT
GTGGAGAAAGTAAATCTCGAGGAGCAGACGCCTGTAGAGAGACAGTAC
CCATTTCAGATTGTCTATAAAGATGGGCTTCTCTATGTCTATGCATCA
AATGAAGAGAGCCGAAGTCAGTGGTTGAAAGCATTACAAAAAGAGATA
AGGGGTAACCCCCACCTGCTGGTCAAGTACCATAGTGGGTTCTTCGTG
GACGGGAAGTTCCTGTGTTGCCAGCAGAGCTGTAAAGCAGCCCCAGGA
TGTACCCTCTGGGAAGCATATGCTAATCTGCATACTGCAGTCAATGAA
GAGAAACACAGAGTTCCCACCTTCCCAGACAGAGTGCTGAAGATACCT
CGGGCAGTTCCTGTTCTCAAAATGGATGCACCATCTTCAAGTACCACT
CTAGCCCAATATGACAACGAATCAAAGAAAAACTATGGCTCCCAGCCA
CCATCTTCAAGTACCAGTCTAGCGCAATATGACAGCAACTCAAAGAAA
ATCTATGGCTCCCAGCCAAACTTCAACATGCAGTATATTCCAAGGGAA
GACTTCCCTGACTGGTGGCAAGTAAGAAAACTGAAAAGTAGCAGCAGC
AGTGAAGATGTTGCAAGCAGTAACCAAAAAGAAAGAAATGTGAATCAC
ACCACCTCAAAGATTTCATGGGAATTCCCTGAGTCAAGTTCATCTGAA
GAAGAGGAAAACCTGGATGATTATGACTGGTTTGCTGGTAACATCTCC
AGATCACAATCTGAACAGTTACTCAGACAAAAGGGAAAAGAAGGAGCA
TTTATGGTTAGAAATTCGAGCCAAGTGGGAATGTACACAGTGTCCTTA
TTTAGTAAGGCTGTGAATGATAAAAAAGGAACTGTCAAACATTACCAC
GTGCATACAAATGCTGAGAACAAATTATACCTGGCAGAAAACTACTGT
TTTGATTCCATTCCAAAGCTTATTCATTATCATCAACACAATTCAGCA
GGCATGATCACACGGCTCCGCCACCCTGTGTCAACAAAGGCCAACAAG
GTCCCCGACTCTGTGTCCCTGGGAAATGGAATCTGGGAACTGAAAAGA
GAAGAGATTACCTTGTTGAAGGAGCTGGGAAGTGGCCAGTTTGGAGTG
GTCCAGCTGGGCAAGTGGAAGGGGCAGTATGATGTTGCTGTTAAGATG
ATCAAGGAGGGCTCCATGTCAGAAGATGAATTCTTTCAGGAGGCCCAG
ACTATGATGAAACTCAGCCATCCCAAGCTGGTTAAATTCTATGGAGTG
TGTTCAAAGGAATACCCCATATACATAGTGACTGAATATATAAGCAAT
GGCTGCTTGCTGAATTACCTGAGGAGTCACGGAAAAGGACTTGAACCT
TCCCAGCTCTTAGAAATGTGCTACGATGTCTGTGAAGGCATGGCCTTC
TTGGAGAGTCACCAATTCATACACCGGGACTTGGCTGCTCGTAACTGC
TTGGTGGACAGAGATCTCTGTGTGAAAGTATCTGACTTTGGAATGACA
AGGTATGTTCTTGATGACCAGTATGTCAGTTCAGTCGGAACAAAGTTT
CCAGTCAAGTGGTCAGCTCCAGAGGTGTTTCATTACTTCAAATACAGC
AGCAAGTCAGACGTATGGGCATTTGGGATCCTGATGTGGGAGGTGTTC
AGCCTGGGGAAGCAGCCCTATGACTTGTATGACAACTCCCAGGTGGTT
CTGAAGGTCTCCCAGGGCCACAGGCTTTACCGGCCCCACCTGGCATCG
GACACCATCTACCAGATCATGTACAGCTGCTGGCACGAGCTTCCAGAA
AAGCGTCCCACATTTCAGCAACTCCTGTCTTCCATTGAACCACTTCGG
GAAAAAGACAAGCATTGAgcggccgc