Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of MRCK alpha [1 - 473]

Enzyme description:- MRCK alpha [1 - 473]

Clone number:- DU 62967

Source:- Recombinant

Expression system:- *E.coli*

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 %

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, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

Assay Buffer:-
50 mM Tris-HCl pH 7.5, 0.1mM EGTA, 10 mM DTT, 10 mM MgAc

Substrate:-
KKRNRTLTV Final concentration: 300 uM

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Clone Data Sheet

MRCK alpha [1 - 473]

Protein	MRCK alpha [1 - 473]
Clone number	DU 62967
Species	Human
Accession number	NM_003607.3
Tags	N-terminal GST
Bacterially 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 pGEX6P-1

Nucleotide Sequence Of Insert

ggatccATGTCTGGAGAAGTGCGTTTGAGGCAGTTGGAGCAGTTTATTT
TGGACGGGCCCGCTCAGACCAATGGGCAGTGCTTCAGTGTGGAGACATT
ACTGGATATACTCATCTGCCTTTATGATGAATGCAATAATTCTCCATTG
AGAAGAGAGAAGAACATTCTCGAATACCTAGAATGGGCTAAACCATTTA
CTTCTAAAGTGAAACAAATGCGATTACATAGAGAAGACTTTGAAATATT
AAAGGTGATTGGTCGAGGAGCTTTTGGGGAGGTTGCTGTAGTAAAACTA
AAAAATGCAGATAAAGTGTTTGCCATGAAAATATTGAATAAATGGGAAA
TGCTGAAAAGAGCTGAGACAGCATGTTTTCGTGAAGAAAGGGATGTATT
AGTGAATGGAGACAATAAATGGATTACAACCTTGCACTATGCTTTCCAG
GATGACAATAACTTATACCTGGTTATGGATTATTATGTTGGTGGGGATT
TGCTTACTCTACTCAGCAAATTTGAAGATAGATTGCCTGAAGATATGGC
TAGATTTTACTTGGCTGAGATGGTGATAGCAATTGACTCAGTTCATCAG
CTACATTATGTACACAGAGACATTAAACCTGACAATATACTGATGGATA
TGAATGGACATATTCGGTTAGCAGATTTTGGTTCTTGTCTGAAGCTGAT
GGAAGATGGAACGGTTCAGTCCTCAGTGGCTGTAGGAACTCCAGATTAT
ATCTCTCCTGAAATCCTTCAAGCCATGGAAGATGGAAAAGGGAGATATG
GACCTGAATGTGACTGGTGGTCTTTGGGGGTCTGTATGTATGAAATGCT
TTACGGAGAAACACCATTTTATGCAGAATCGCTGGTGGAGACATACGGA
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ATGTGTCTGAAAATGCTAAGGATCTTATTCGAAGGCTCATTTGTAGCAG
AGAACATCGACTTGGTCAAAATGGAATAGAAGACTTTAAGAAACACCCA
TTTTTCAGTGGAATTGATTGGGATAATATTCGGAACTGTGAAGCACCTT
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GCATTTTCTGGCCACCATCTGCCATTTGTTGGTTTTACATATACTAGTA
GCTGTGTACTTTCTGATCGGAGCTGTTTAAGAGTTACGGCTGGTCCCAC
CTCACTGGATCTTGATGTTAATGTTCAGAGGACTCTAGACAACAACTTA
GCAACTGAAGCTTATGAAAGAAGAATTAAGCGCCTTGAGCAAGAAAAAC
TTGAACTCAGTAGAAAACTTCAAGAGTCAACACAGACTGTCCAAGCTCT
GCAGtgagcggccgc