Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of MRCK beta [1 - 473]

Enzyme description:- MRCK beta [1 - 473]

Clone number:- DU 27777

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH-Sepharose

Calculated molecular mass:-
Monoisotopic 81, 377.68 daltons
Average Mass 81, 430.17 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.16

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 beta [1 - 473]

Protein	MRCK beta [1 - 473]
Clone number	DU 27777
Species	Human
Accession number	NM_006035.3
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MSAKVRLKKLEQLL** **LDGPWRNESALSVETLLDVLVCLYTECSHSALRRDKYVAEFLEWAKPFT** **QLVKEMQLHREDFEIIKVIGRGAFGEVAVVKMKNTERIYAMKILNKWEM** **LKRAETACFREERDVLVNGDCQWITALHYAFQDENHLYLVMDYYVGGDL** **LTLLSKFEDKLPEDMARFYIGEMVLAIDSIHQLHYVHRDIKPDNVLLDV** **NGHIRLADFGSCLKMNDDGTVQSSVAVGTPDYISPEILQAMEDGMGKYG** **PECDWWSLGVCMYEMLYGETPFYAESLVETYGKIMNHEERFQFPSHVTD** **VSEEAKDLIQRLICSRERRLGQNGIEDFKKHAFFEGLNWENIRNLEAPY** **IPDVSSPSDTSNFDVDDDVLRNTEILPPGSHTGFSGLHLPFIGFTFTTE** **SCFSDRGSLKSIMQSNTLTKDEDVQRDLEHSLQMEAYERRIRRLEQEKL** **ELSRKLQESTQTVQSLHG**
Native sequence	Amino acids M1 – G473 (end residue is T1711) of human MRCK beta. 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

ggatccATGTCGGCCAAGGTGCGGCTCAAGAAGCTGGAGCAGCTGCTCC
TGGACGGGCCCTGGCGCAACGAGAGCGCCCTGAGCGTGGAAACGCTGCT
CGACGTGCTCGTCTGCCTGTACACCGAGTGCAGCCACTCGGCCCTGCGC
CGCGACAAGTACGTGGCCGAGTTCCTCGAGTGGGCTAAACCATTTACAC
AGCTGGTGAAAGAAATGCAGCTTCATCGAGAAGACTTTGAAATAATTAA
AGTAATTGGAAGAGGTGCTTTTGGTGAGGTTGCTGTTGTCAAAATGAAG
AATACTGAACGAATTTATGCAATGAAAATCCTCAACAAGTGGGAGATGC
TGAAAAGAGCAGAGACCGCGTGCTTCCGAGAGGAGCGCGATGTGCTGGT
GAACGGCGACTGCCAGTGGATCACCGCGCTGCACTACGCCTTTCAGGAC
GAGAACCACCTGTACTTAGTCATGGATTACTATGTGGGTGGTGATTTAC
TGACCCTGCTCAGCAAATTTGAAGACAAGCTTCCGGAAGATATGGCGAG
GTTCTACATTGGTGAAATGGTGCTGGCCATTGACTCCATCCATCAGCTT
CATTACGTGCACAGAGACATTAAACCTGACAATGTCCTTTTGGACGTGA
ATGGTCATATCCGCCTGGCTGACTTTGGATCATGTTTGAAGATGAATGA
TGATGGCACTGTGCAGTCCTCCGTGGCCGTGGGCACACCTGACTACATC
TCGCCGGAGATCCTGCAGGCGATGGAGGACGGCATGGGCAAATACGGGC
CTGAGTGTGACTGGTGGTCTCTGGGTGTCTGCATGTATGAGATGCTCTA
TGGAGAAACGCCGTTTTATGCGGAGTCACTCGTGGAGACCTATGGGAAG
ATCATGAACCATGAAGAGCGATTCCAGTTCCCATCCCATGTCACGGATG
TATCTGAAGAAGCGAAGGACCTCATCCAGAGACTGATCTGCAGTAGAGA
ACGCCGGCTGGGGCAGAATGGAATAGAGGATTTCAAAAAGCATGCGTTT
TTTGAAGGTCTAAATTGGGAAAATATACGAAACCTAGAAGCACCTTATA
TTCCTGATGTGAGCAGTCCCTCTGACACATCCAACTTCGACGTGGATGA
CGACGTGCTGAGAAACACGGAAATATTACCTCCTGGTTCTCACACAGGC
TTTTCTGGATTACATTTGCCATTCATTGGTTTTACATTCACAACGGAAA
GCTGTTTTTCTGATCGAGGCTCTCTGAAGAGCATAATGCAGTCCAACAC
ATTAACCAAAGATGAGGATGTGCAGCGGGACCTGGAGCACAGCCTGCAG
ATGGAAGCTTACGAGAGGAGGATTCGGAGGCTGGAACAGGAGAAGCTGG
AGCTGAGCAGGAAGCTGCAAGAGTCCACCCAGACCGTGCAGTCCCTCCA
CGGCtgagcggccgc