Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active MSPK1 [1 - 305]

Enzyme description:- MSPK1 [1 - 305]

Clone number:- DU 11629

Source:- Recombinant

Expression system:- *E.coli*, co-expressed

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 61, 470.18 daltons
Average Mass 61, 510.10 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.10

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:-
Myelin Basic Protein Final concentration: 1 mg/ml

Division of Signal Transduction Therapy

Clone Data Sheet

MPSK1 [1 - 305]

Protein	MPSK1 [1 - 305]
Clone number	DU 11629
Species	Human
Accession number	CR407675
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MGHALCVCS** **RGTVIIDNKRYLFIQKLGEGGFSYVDLVEGLHDGHFYALKRILCHEQQ** **DREEAQREADMHRLFNHPNILRLVAYCLRERGAKHEAWLLLPFFKRGT** **LWNEIERLKDKGNFLTEDQILWLLLGICRGLEAIHAKGYAHRDLKPTN** **ILLGDEGQPVLMDLGSMNQACIHVEGSRQALTLQDWAAQRCTISYRAP** **ELFSVQSHCVIDERTDVWSLGCVLYAMMFGEGPYDMVFQKGDSVALAV** **QNQLSIPQSPRHSSALWQLLNSMMTVDPHQRPHIPLLLSQLEALQPPA** **PGQHTTQI**
Native sequence	Amino acids M1 – I305 of human MPSK1. 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	*Bam*H1 and *Not*1 sites of pGEX-6P-2

Nucleotide sequence of insert

ggatccATGGGCCACGCGCTGTGTGTCTGCTCTCGGGGAACTGTCATC
ATTGACAATAAGCGCTACCTCTTCATCCAGAAACTGGGGGAGGGTGGG
TTCAGCTATGTGGACCTAGTGGAAGGGTTACATGATGGACACTTCTAC
GCCCTGAAGCGAATCCTGTGTCACGAGCAGCAGGACCGGGAGGAGGCC
CAGCGAGAAGCCGACATGCATCGCCTCTTCAATCACCCCAACATCCTT
CGCCTCGTGGCTTACTGTCTGAGGGAACGGGGTGCTAAGCATGAGGCC
TGGCTGCTGCTACCATTCTTCAAGAGAGGTACGCTGTGGAATGAGATA
GAAAGGCTGAAGGACAAAGGCAACTTCCTGACCGAGGATCAAATCCTT
TGGCTGCTGCTGGGGATCTGCAGAGGCCTTGAGGCCATTCATGCCAAG
GGTTATGCCCACAGAGACTTGAAGCCCACCAATATATTGCTTGGAGAT
GAGGGGCAGCCAGTTTTAATGGACTTGGGTTCCATGAATCAAGCATGC
ATCCATGTGGAGGGCTCCCGCCAGGCTCTGACCCTGCAGGACTGGGCA
GCCCAGCGGTGCACCATCTCCTACCGAGCCCCAGAGCTCTTCTCTGTG
CAGAGTCACTGTGTCATCGATGAGCGGACTGATGTCTGGTCCCTAGGC
TGCGTGCTATATGCCATGATGTTTGGGGAAGGCCCTTATGACATGGTG
TTCCAAAAGGGTGACAGTGTGGCCCTTGCTGTGCAGAACCAACTCAGC
ATCCCACAAAGCCCCAGGCATTCTTCAGCATTGTGGCAGCTCCTGAAC
TCGATGATGACCGTGGACCCGCATCAGCGTCCTCACATTCCTCTCCTC
CTCAGTCAGCTGGAGGCGCTGCAGCCCCCAGCTCCTGGCCAACATACT
ACCCAAATCtgagcggccgc