Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active MAP3K19 [941 - 1328]

Enzyme description:- MAP3K19 [941 - 1328]

Clone number:- DU 62286

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N–terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 70, 263.51 daltons
Average Mass 70, 309.24 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.14

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, 10 mM DTT, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70 °C

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

Substrate:-
Myelin Basic Protein Final concentration: 0.33 mg/ml

Clone Data Sheet

MAP3K19 [941 - 1328]

Protein	MAP3K19 [941 - 1328]
Clone number	DU 62286
Species	Human
Accession number	NM_025052.4
Tags	N-terminal GST
Baculovirus expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNK KFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKE RAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKM FEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKL VCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSD LEVLFQGPLGS**MFGKTLSGTNSISQEIMDSVNNEELTDELLGCL** **AAELLALDEKDNNSCQKMANETDPENLNLVLRWRGSTPKEMGRE** **TTKVKIQRHSSGLRIYDREEKFLISNEKKIFSENSLKSEEPILW** **TKGEILGKGAYGTVYCGLTSQGQLIAVKQVALDTSNKLAAEKEY** **RKLQEEVDLLKALKHVNIVAYLGTCLQENTVSIFMEFVPGGSIS** **SIINRFGPLPEMVFCKYTKQILQGVAYLHENCVVHRDIKGNNVM** **LMPTGIIKLIDFGCARRLAWAGLNGTHSDMLKSMHGTPYWMAPE** **VINESGYGRKSDIWSIGCTVFEMATGKPPLASMDRMAAMFYIGA** **HRGLMPPLPDHFSENAADFVRMCLTRDQHERPSALQLLKHSFLE** **RSH**
Native sequence	Amino acids M941 – H1328 (end residue) of human MAP3K19. Residue M232 of the fusion protein is equivalent to M941 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*1sites of pFastBac Dual.

Nucleotide sequence of insert

ggatccATGTTTGGAAAAACCTTAAGTGGCACAAATTCAATTTCC
CAAGAAATTATGGACTCTGTAAATAATGAAGAATTGACAGATGAA
CTATTAGGTTGTCTAGCTGCAGAATTATTAGCTCTTGATGAGAAA
GATAACAACTCTTGCCAAAAAATGGCAAATGAAACAGATCCTGAA
AACCTAAATCTTGTCCTCAGATGGAGAGGAAGTACCCCAAAAGAA
ATGGGCAGAGAGACAACAAAAGTCAAAATACAGAGGCATAGTAGT
GGGCTCAGGATATATGACAGGGAGGAGAAATTTCTCATCTCAAAT
GAAAAGAAGATATTTTCTGAAAATAGTTTAAAGTCTGAAGAACCT
ATCCTATGGACCAAGGGTGAGATTCTTGGAAAGGGAGCCTACGGC
ACAGTATACTGTGGTCTCACTAGTCAAGGACAGCTAATAGCTGTA
AAACAGGTGGCTTTGGATACCTCTAATAAATTAGCTGCTGAAAAG
GAATACCGGAAACTACAGGAAGAAGTAGATTTGCTCAAAGCACTG
AAACATGTCAACATTGTGGCCTATTTGGGGACATGCTTGCAAGAG
AACACTGTGAGCATTTTCATGGAGTTTGTTCCTGGTGGCTCAATC
TCTAGTATTATAAACCGTTTTGGGCCATTGCCTGAGATGGTGTTC
TGTAAATATACGAAACAAATACTTCAAGGTGTTGCTTATCTCCAT
GAGAACTGTGTGGTACATCGCGATATCAAAGGAAATAATGTTATG
CTCATGCCAACTGGAATAATAAAGCTGATTGACTTTGGCTGTGCC
AGGCGTTTGGCCTGGGCAGGTTTAAATGGCACCCACAGTGACATG
CTTAAGTCCATGCATGGGACTCCATATTGGATGGCCCCAGAAGTC
ATCAATGAGTCTGGCTATGGACGGAAATCAGATATCTGGAGCATT
GGTTGTACTGTGTTTGAGATGGCTACAGGGAAGCCTCCACTGGCT
TCCATGGACAGGATGGCCGCCATGTTTTACATCGGAGCACACCGA
GGGCTGATGCCTCCTTTACCAGACCACTTCTCAGAAAATGCAGCA
GACTTTGTGCGCATGTGCCTGACCAGGGACCAGCATGAGCGACCT
TCTGCTCTCCAGCTCCTGAAGCACTCCTTCTTGGAGAGAAGTCAC
tgagcggccgc