

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active MAP4K5 [2 – 846]

<u>Enzyme description:-</u>	MAP4K5 [2 – 846]
<u>Clone number:-</u>	DU 38642
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal GST
<u>Purification method:-</u>	Glutathione Sepharose

Calculated molecular mass:-

Monoisotopic 121, 694.94 daltons
Average Mass 121, 772.96 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.91

Purity:- 75 %

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 °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

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Clone Data Sheet

MAP4K5 [2 – 846]

Protein MAP4K5 [2 - 846]

Clone number DU 38642

Species Human

Accession number NM_006575.4

Tags N-terminal GST

**Baculovirus
expressed protein**

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKK
FELGLEFPNLPYYIDGDVKLTSMAIIRYIADKHNMLGGCPKERA
EISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPPEMLKMFED
RLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFK
KRIEAIPOIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLEVL
FQGPLGSEAPLRPAADILRRNPQDYELVQRVGSGTYGDVYKARNV
HTGELAAVKI IKLEPGDDFSLIQQEIFMVKECKHCNIVAYFGSYL
SREKLWICMEYCGGSLQDIYHVTGPLSELOIAYVCRETLOGLAY
LHTKGKMRDIKGANILLTDHGDKLADFGVAAKITATIAKRKSF
IGTPYWMAPEVAAVEKNGGYNQLCDIWA VGITAIELGELQPPMFD
LHPMRALFLMSKSNFQPPKLDKTKWSSTFHNFKIALTKNPKKR
PTAERLLTHTFVAQPGLSRALAVELLDKVNNPDNHAHYTEADDD
FEPHAIIRHTIRSTNRNARAERTASEINFDKLQFEPPLRKETEAR
DEMGLSSDPNFMLOWNPFVDGANTGKSTSKRAIPPLPKPRISS
YPEDNFPDEEKASTIKHCPDSESRAPQILRRQSSPSCGPVAETSS
IGNGDGISKLMSENTEGSAQAPQLPRKKDKRDFPKPAINGLPPTP
KVLMGACFSKVFDGCPLKINCATSWIHPDTKDQYIIFGTEDGIYT
LNLNELYEATMEQLFPRKCTWLYVINNTLMSLSEGKTFQLYSHNL
IALFEHAKKPGLAAHIQTHRFPDRILPRKFALTTKIPDTKGCHKC
CIVRNPYTGHKYLCGALQSGIVLLQWYEPMQKFMLIKHFDPLPS
PLNVFEMLVIPEQEYPMVCVAISKGTESNOVVQFETINLNSASSW
FTEIGAGSQQLDSIHVTQLERDTVLVCLDKFVKIVNLQGLKSSK
KLASELSFDFRIESVVCLQDSVLAFWKHGMQGSFKSDEVTOEIS
DETRVFRLLGSDRVVLESRPTENPTAHSNLYILAGHENSY

Native sequence Amino acids E2 – Y846 (end) of human MAP4K5.
Residue E232 of the fusion protein is equivalent to E2 of the native enzyme. The GST tag is located at residues 1 - 220.

The following amino acid substitution is present
H – Y, where H542 of the native enzyme is **Y772** of the fusion protein

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Protease cleavage PreScission (LEVLFQGP) residues 221 - 228

Cloning sites *Bam*H1 sites of pFB-GST

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Nucleotide sequence of insert

ggatccGAGGCCCGCTGCGGCCTGCCGCGGACATCCTGAGGCGG
AACCCGCAGCAGGACTACGAACTCGTCCAGAGGGTCGGCAGCGGC
ACCTACGGGGACGTCTATAAGGCCAGAAATGTACACACAGGAGAG
CTGGCTGCAGTAAAAATCATTAATTTGGAGCCTGGAGATGATTTT
TCTTTGATTCAACAAGAAATATTTATGGTTAAAGAATGTAAACAT
TGTAACATCGTTGCCTACTTTGGGAGTTATCTTAGTCGGGAAAAA
CTATGGATTTGTATGGAATACTGTGGTGGCGGATCACTCCAAGAT
ATTTACCATGTTACTGGACCATTATCAGAATTGCAAATAGCCTAT
GTATGCAGAGAAACCTTACAGGGTCTTGCCTATTTGCATACTAAA
GGCAAATGCATAGAGATATCAAAGGTGCTAATATTTTATTGACA
GACCATGGCGATGTAAAAATTAGCTGACTTTGGTGTGGCTGCAAAA
ATAACAGCTACCATTGCAAACGAAAATCTTTCATTGGCACCCCT
TACTGGATGGCCCCAGAAGTTGCAGCAGTAGAGAAGAATGGTGGC
TACAACCAACTCTGTGATATCTGGGCAGTAGGAATAACAGCAATT
GAACTTGGAGAACTTCAGCCACCTATGTTTGATCTCCACCCAATG
AGGGCTCTCTTCTTAATGTCAAAAAGTAATTTTCAGCCTCCAAAA
CTAAAGGACAAAACAAAATGGTCATCAACATTCCATAATTTTGTC
AAAATAGCACTAACCAAAAACCCAAAAAAAAGACCAACTGCTGAA
AGACTTCTGACTCACACTTTTGTGTCACAGCCAGGTCTCTCTAGA
GCCCTAGCAGTTGAACTGTTAGACAAAGTGAACAATCCAGATAAC
CACGCACATTACACTGAAGCAGATGACGATGACTTTGAGCCCCAT
GCAATCATTCGTCATAACCATTAGATCTACAAACAGGAATGCCAGA
GCTGAACGGACAGCTTCAGAAATAAATTTTGACAAATTACAATTT
GAACCTCCTCTGAGAAAAGAAACAGAAGCACGAGATGAAATGGGA
TTGTCATCAGACCCAAATTTTCATGTTACAGTGGAATCCTTTTGTT
GATGGTGCAAATACTGGCAAATCAACCTCAAACCGTGCAATACCA
CCTCCCCTACCTCCTAAGCCAAGGATAAGCAGTTACCCTGAAGAC
AACTTTCCGGATGAAGAAAAGCATCAACCATAAAACATTGTCCCT
GATTCAGAAAGCAGAGCTCCCCAAATTCTCAGAAGACAGAGTAGC
CCAAGTTGTGGGCCTGTGGCAGAGACTTCTTCTATTGGAAATGGT
GATGGTATTTCAAACCTGATGAGTGAAAATACAGAAGGATCAGCA
CAAGCACCACAGTTACCACGAAAAAAGGACAAACGAGACTTCCCT
AAACCAGCCATCAATGGCCTTCCACCCACCCCAAAGTTCTGATG
GGAGCATGCTTTTCAAAGTTTTTTGATGGCTGTCCTTTGAAAATT
AATTGTGCAACATCCTGGATACATCCTGATACAAAAGATCAGTAC
ATTATTTTTGGAACCTGAAGATGGTATTTACACACTGAATCTCAAT
GAGCTATATGAGGCAACGATGGAACAGTTATTTCCACGGAAGTGT
ACTTGGCTGTATGTTATCAATAATACTTTAATGTCATTATCAGAA
GGAAAAACCTTTCAGCTCTACTCTCACAATCTTATAGCTTTGTTT
GAACATGCCAAAAAACAGGATTAGCTGCCCATATTCAAACTCAC
AGGTTTCCAGACCGAATACTACCAAGAAAATTCGCTTTAACAACA
AAGATTCCCTGATACAAAAGGCTGCCACAAATGTTGCATAGTCAGA

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AACCCTTACACGGGACATAAATACCTCTGTGGAGCTTTACAGTCT
GGAATTGTTTTACTTCAGTGGTATGAGCCAATGCAGAAATTCATG
TTGATAAAGCACTTTGATTTTCCTTTGCCAAGTCCTTTGAATGTT
TTTGAAATGCTGGTGATACCTGAACAGGAATACCCTATGGTCTGT
GTAGCTATTAGCAAAGGCACTGAATCGAATCAGGTAGTTCAGTTT
GAGACAATCAATTTGAACTCTGCATCTTCATGGTTTTACAGAAAT
GGTGCAGGCAGTCAGCAGTTAGATTCCATTCATGTAACACAGTTG
GAGAGAGATACCGTTTTAGTGTGTTTAGACAAATTTGTGAAAAT
GTAAATCTACAAGGAAAATTAATAATCAAGTAAGAACTGGCCTCT
GAGTTAAGTTTTGATTTTCGCATTGAATCTGTAGTATGCCTTCAA
GACAGTGTGTTGGCTTTCTGGAAACATGGGATGCAGGGTAAAAGC
TTCAAGTCAGATGAGGTTACCCAGGAGATTTTCAGATGAAACAAGA
GTTTTCCGCTTATTAGGATCAGACAGGGTTGTCGTTTTGGAAAGT
AGGCCAACAGAAAATCCTACTGCACACAGCAATCTCTACATCTTG
GCTGGACATGAAAATAGTTACTaaggatcc