

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active MEKK1 [877 - 1563]

<u>Enzyme description:-</u>	MEKK1 [877 - 1563]
<u>Clone number:-</u>	DU 819
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	<i>E.coli</i>
<u>Tag:-</u>	N-terminal GST and C-terminal His(6)
<u>Purification method:-</u>	GSH sepharose and Ni ²⁺ -NTA agarose

Calculated molecular mass:-

Monoisotopic 102,692.25 daltons
Average Mass 102,757.60 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.87

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

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Clone Data Sheet

MEKK1 [877 - 1563]

<u>Protein</u>	MEKK1 [877 - 1563]
<u>Clone number</u>	DU 819
<u>Species</u>	Human
<u>Accession number</u>	XM_042066
<u>Tags</u>	N-terminal GST and C-terminal His(6)
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGL EFPNLPYYIDGDVKTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVL DIRYGVSRIAYSKDFETLKVDLFLSKLPEMLKMFEDRLCHKTYLNGDHVTH PDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSSKYIA WPLQGWQATFGGDHPPKSDLEVLFOGPLGSMVTTVPHVFSKLEMLSVS SSTHFTRMRRRLMAIADEVEIAEAIQLGVEDTLDGQODSFLQASVPNNYL ETTENSPECTIHLEKTGKGLCATKLSASSEDISERLASISVGPSSSTTT TTTTEQPKPMVQTKGRPHSQCLNSSPLSHHSQLMFPALSTPSSSTPSVPA GTATDVSKHRLQGFIPCRIPASAPQTQRFSLQFHRNCPENKDSKLSPV FTQSRPLPSSNIHRPKPSRPTPGNTSKQGDPSKNSMTLDLNSSSKCDDSF GCSSNSSNAVIPSETVFTPVEEKCRLDVNTLNSSIEDLLEASMPSSDT TVTFFKSEVAVLSPEKAENDDTYKDDVNHNOCKCEKMEAEIEEEALAIAMAM SASQDALPIVPQLQVENGEDI I I I QODTPETLPGHTKAKOPYREDTEWLK GQQIGLGAFSSCYQAQDVGTGLMAVKQVTVRNTSSEQEEVVEALREEI RMMSHLNHPNIIRMLGATCEKSNYNLFIEWMAGGSVAHLLSKYGAFKESV VINYTEQLLRGLSYLHENQI IHRDVKGANLLIDSTGQRLRIADFGAAARL ASKGTGAGEFQGQLLGTIAFMAPEVLRGQOYGRSCDVWSVGCAL IEMACA KPPWNAEKHSNHLALIFKIASATTAPSI PSHLSPLGRDVALRCLELQPQD RPPSRELLKHPVFRTTWHHHHHH</p>
<u>Native sequence</u>	<p>Amino acids M877 – W1563 (end) of human MEKK1. Residue M232 of the fusion protein is equivalent to M877 of human MEKK1. The GST tag is located at residues 1 – 220 and the His(6) tag is located at residues 918 – 923.</p> <p>The fusion protein lacks a threonine, as compared to the native sequence [T993].</p>
<u>Protease cleavage</u>	PreScission site (<u>LEVLFQGPL</u>) at residues 221 – 229

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Cloning sites

*Bam*H1 and *Eco*R1 site of pGEX6P-1

Nucleotide sequence of insert

GGATCCATGGTTACTACAGTACCCCATGTGTTTTCAAACCTGTTAGAAAT
GCTGAGTGTTCAGTTCCACTCACTTCACCAGGATGCGTCGCCGTTTGA
TGGCTATTGCAGATGAGGTGGAAATTGCCGAAGCCATCCAGTTGGGCGTA
GAAGACACTTTGGATGGTCAACAGGACAGCTTCTTGCAGGCATCTGTTCC
CAACAACCTATCTGGAAACCACAGAGAACAGTTCCCCTGAGTGCACAATCC
ATTTAGAGAAAACCTGGAAAAGGATTATGTGCTACAAAATTGAGTGCCAGT
TCAGAGGACATTTCTGAGAGACTGGCCAGCATTTCAGTAGGACCTTCTAG
TTCAACAACAACAACAACAACAACAGAGCAACCAAAGCCAATGGTTCAA
CAAAGGCAGACCCACAGTCAGTGTGGAACTCCTCTCCTTTATCTCAT
CATTCCCAATTAATGTTTCCAGCCTTGTCAACCCCTTCTTCTTCTACCCC
ATCTGTACCAGCTGGCACTGCAACAGATGTCTCTAAGCATAGACTTCAGG
GATTCATTCCCTGCAGAATACCTTCTGCATCTCCTCAAACACAGCGCAAG
TTTTCTCTACAATTCCACAGAACTGTCTGAAAACAAAGACTCAGATAA
ACTTTCCCAGTCTTTACTCAGTCAAGACCCTTGCCCTCCAGTAACATAC
ACAGGCCAAAGCCATCTCGACCTACCCAGGTAATACAAGTAAACAGGGA
GATCCCTCAAAAAATAGCATGACACTTGATCTGAACAGTAGTTCCAAATG
TGATGACAGCTTTGGCTGTAGCAGCAATAGTAGTAATGCTGTTATACCCA
GTGACGAGACAGTGTTCACCCAGTAGAGGAGAAATGCAGATTAGATGTC
AATACAGAGCTCAACTCCAGTATTGAGGACCTTCTTGAAGCATCTATGCC
TTCAAGTGATACAACAGTAACTTTTAAGTCAGAAGTTGCTGTCTGTCTC
CTGAAAAGGCTGAAAATGATGATACCTACAAAGATGATGTGAATCATAAT
CAAAGTGCAAAGAGAAGATGGAAGCTGAAGAAGAAGAAGCTTTAGCAAT
TGCCATGGCAATGTCAGCGTCTCAGGATGCCCTCCCATAGTTCCTCAGC
TGCAGGTTGAAAATGGAGAAGATATCATCATTATTC AACAGGATACACCA
GAGACTCTACCAGGACATACC AAAGCAAACAACCGTATAGAGAAGACAC
TGAATGGCTGAAAGGTCAACAGATAGGCCTTGGAGCATTTTCTTCTTGTT
ATCAGGCTCAAGATGTGGGAACTGGAAC TTTAATGGCTGTTAAACAGGTG
ACTTATGTCAGAAACACATCTTCTGAGCAAGAAGAAGTAGTAGAAGCACT
AAGAGAAGAGATAAGAATGATGAGCCATCTGAATCATCCAAACATCATTA
GGATGTTGGGAGCCACGTGTGAGAAGAGCAATTACAATCTCTTCATTGAA
TGGATGGCAGGGGATCGGTGGCTCATT TGCTGAGTAAATATGGAGCCTT
CAAAGAATCAGTAGTTATTA ACTACTGAACAGTTACTCCGTGGCCTTT
CGTATCTCCATGAAAACCAAATCATT CACAGAGATGTCAAAGGTGCCAAT
TTGCTAATTGACAGCACTGGT CAGAGACTAAGAATTGCAGATTTTGGAGC
TGCAGCCAGGTTGGCATCAAAGGAACTGGTGCAGGAGAGTTTCAGGGAC
AATTACTGGGGACAATTGCATTTATGGCACCTGAGGTACTAAGAGGTCAA
CAGTATGGAAGGAGCTGTGATGTATGGAGTGTGGCTGTGCTATTATAGA
AATGGCTTGTGCAAACCAACATGGAATGCAGAAAAACCTCCAATCATC
TTGCTTTGATATTTAAGATTGCTAGTGCAACTACTGCTCCATCGATCCCT
TCACATTTGTCTCCTGGTTTACGAGATGTGGCTCTTCGTTGTTTAGAACT
TCAACCTCAGGACAGACCTCCATCAAGAGAGCTACTGAAGCATCCAGTCT
TTCGTA CTACATGGCATCATCACCATCACCATTGAgaattcgc