Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active MKK1 [1 - 393]

Enzyme description:- MKK1 [1 - 393]

Clone number:- DU 911

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST and C-terminal His(6)

Purification method:- GSH Sepharose followed by Ni^{2+}-NTA agarose

Expression level:- 2-3 mg/L

Calculated molecular mass:- 70, 909 daltons

Purity:- >80 %

Activation protocol:-

MKK1 (2.5 µM) is activated in 50 mM Tris-HCl pH 7.5, 0.1mM EGTA, 0.1 % 2-mercaptoethanol, 10 mM MgAc, 0.1mMATP, with 50 units/ml GST-cRaf- DD [DU 811] at 30 °C for 30 min. Following activation, the active enzyme is repurified on Ni^{2+}-NTA agarose.

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM sucrose, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

Assay:-
Two step assay in which MKK1 activates unactive MAPK2/ERK2 [DU 650 or DU 1844]. Activity of MAPK2/ERK2 is then assayed against myelin basic protein as substrate (final concentration of 0.3 mg/ml), in the standard filter binding assay.

Assay buffer:-
50 mM Tris-HCl pH 7.5, 0.1 % 2-mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc

Specific activity range:- 4000 – 8000 U/mg

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Clone Data Sheet

MKK1 [2 - 393]

Protein	MKK1 [2 - 393]
Clone number	DU 911
Species	Human
Accession number	L05624
Tags	N-terminal GST and C-terminal His(6)
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**PKKKPTPIQ** **LNPAPDGSAVNGTSSAETNLEALQKKLEELELDEQQRKRLEAFLTQKQ** **KVGELKDDDFEKISELGAGNGGVVFKVSHKPSGLVMARKLIHLEIKPA** **IRNQIIRELQVLHECNSPYIVGFYGAFYSDGEISICMEHMDGGSLDQV** **LKKAGRIPEQILGKVSIAVIKGLTYLREKHKIMHRDVKPSNILVNSRG** **EIKLCDFGVSGQLIDSMANSFVGTRSYMSPERLQGTHYSVQSDIWSMG** **LSLVEMAVGRYPIPPPDAKELELMFGCQVEGDAAETPPRPRTPGRPLS** **SYGMDSRPPMAIFELLDYIVNEPPPKLPSGVFSLEFQDFVNKCLIKNP** **AERADLKQLMVHAFIKRSDAEEVDFAGWLCSTIGLNQPSTPTHAAGV**HHHHHH
Native sequence	Amino acids P2 – V393 (end) of human MEK1. Residue P232 of the fusion protein is equivalent to P2 of the native enzyme. The GST tag is located at residues 1 – 220 and the His(6) is located at residues 624-629.
Protease cleavage	Prescission site (LEVLFQGPL) at residues 221 - 229
Cloning sites	*Bam*H1 site of pGEX-6P-1

Nucleotide sequence of insert

GGATCCCCCAAGAAGAAGCCGACGCCCATCCAGCTGAACCCGGCCCCC
GACGGCTCTGCAGTTAACGGGACCAGCTCTGCGGAGACCAACTTGGAG
GCCTTGCAGAAGAAGCTGGAGGAGCTAGAGCTTGATGAGCAGCAGCGA
AAGCGCCTTGAGGCCTTTCTTACCCAGAAGCAGAAGGTGGGAGAACTG
AAGGATGACGACTTTGAGAAGATCAGTGAGCTGGGGGCTGGCAATGGC
GGTGTGGTGTTCAAGGTCTCCCACAAGCCTTCTGGCCTGGTCATGGCC
AGAAAGCTAATTCATCTGGAGATCAAACCCGCAATCCGGAACCAGATC
ATAAGGGAGCTGCAGGTTCTGCATGAGTGCAACTCTCCGTACATCGTG
GGCTTCTATGGTGCGTTCTACAGCGATGGCGAGATCAGTATCTGCATG
GAGCACATGGATGGAGGTTCTCTGGATCAAGTCCTGAAGAAAGCTGGA
AGAATTCCTGAACAAATTTTAGGAAAAGTTAGCATTGCTGTAATAAAA
GGCCTGACATATCTGAGGGAGAAGCACAAGATCATGCACAGAGATGTC
AAGCCCTCCAACATCCTAGTCAACTCCCGTGGGGAGATCAAGCTCTGT
GACTTTGGGGTCAGCGGGCAGCTCATCGACTCCATGGCCAACTCCTTC
GTGGGCACAAGGTCCTACATGTCGCCAGAAAGACTCCAGGGGACTCAT
TACTCTGTGCAGTCAGACATCTGGAGCATGGGACTGTCTCTGGTAGAG
ATGGCGGTTGGGAGGTATCCCATCCCTCCTCCAGATGCCAAGGAGCTG
GAGCTGATGTTTGGGTGCCAGGTGGAAGGAGATGCGGCTGAGACCCCA
CCCAGGCCAAGGACCCCCGGGAGGCCCCTTAGCTCATACGGAATGGAC
AGCCGACCTCCCATGGCAATTTTTGAGTTGTTGGATTACATAGTCAAC
GAGCCTCCTCCAAAACTGCCCAGTGGAGTGTTCAGTCTGGAATTTCAA
GATTTTGTGAATAAATGCTTAATAAAAAACCCCGCAGAGAGAGCAGAT
TTGAAGCAACTCATGGTTCATGCTTTTATCAAGAGATCTGATGCTGAG
GAAGTGGATTTTGCAGGTTGGCTCTGCTCCACCATCGGCCTTAACCAG
CCCAGCACACCAACCCATGCTGCTGGCGTCCATCATCACCATCACCAT
TAAGGATC