Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active MKK2 [2 - 400]

Enzyme description:- MKK2 [2 - 400]

Clone number:- DU 727

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:- 1 mg/L

Calculated molecular mass:- 71, 893 daltons

Purity:- >80 %

Activation protocol:-
MKK2 (4 µM) is activated in 50 mM Tris-HCl pH 7.5, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 10 mM MgAc, 0.1 mM ATP, with 100 µg/ml His-MEKK1 [DU 1847] at 30 °C for 30 min. Following activation, MKK2 is re-purified by GSH Sepharose chromatography.

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:-
Two step assay in which MKK2 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:- 5000 – 10000 U/mg

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Clone Data Sheet

MKK2 [2 - 400]

Protein	MKK2 [2 - 400]
Clone number	DU 727
Species	Human
Accession number	NM_030662
Tags	N-terminal GST and C-terminal His(6)
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**LARRKPVLP** **ALTINPTIAEGPSPTSEGASEANLVDLQKKLEELELDEQQKKRLEAFL** **TQKAKVGELKDDDFERISELGAGNGGVVTKVQHRPSGLIMARKLIHLE** **IKPAIRNQIIRELQVLHECNSPYIVGFYGAFYSDGEISICMEHMDGGS** **LDQVLKEAKRIPEEILGKVSIAVLRGLAYLREKHQIMHRDVKPSNILV** **NSRGEIKLCDFGVSGQLIDSMANSFVGTRSYMAPERLQGTHYSVQSDI** **WSMGLSLVELAVGRYPIPPPDAKELEAIFGRPVVDGEEGEPHSISPRP** **RPPGRPVSGHGMDSRPAMAIFELLDYIVNEPPPKLPNGVFTPDFQEFV** **NKCLIKNPAERADLKMLTNHTFIKRSEVEEVDFAGWLCKTLRLNQPGT** **PTRTAV**HHHHHH
Native sequence	Amino acids L2 – V400 (end) of human MKK2. Residue L232 of the fusion protein is equilivalent to L2 of the native enzyme. The GST tag is located at residues 1 – 220 and the His(6) is located at residues 631 – 636.
Protease cleavage	PreScission site (LEVLFQGPL) at residues 221 – 229
Cloning sites	*Bam*H1 and *Eco*R1 site of pGEX6P-1

Nucleotide sequence of insert

GGATCCCTGGCCCGGAGGAAGCCGGTGCTGCCGGCGCTCACCATCAAC
CCTACCATCGCCGAGGGCCCATCCCCTACCAGCGAGGGCGCCTCCGAG
GCAAACCTGGTGGACCTGCAGAAGAAGCTGGAGGAGCTGGAACTTGAC
GAGCAGCAGAAGAAGCGGCTGGAAGCCTTTCTCACCCAGAAAGCCAAG
GTTGGCGAACTCAAAGACGATGACTTCGAAAGGATCTCAGAGCTGGGC
GCGGGCAACGGCGGGGTGGTCACCAAAGTCCAGCACAGACCCTCGGGC
CTCATCATGGCCAGGAAGCTGATCCACCTTGAGATCAAGCCGGCCATC
CGGAACCAGATCATCCGCGAGCTGCAGGTCCTGCACGAATGCAACTCG
CCGTACATCGTGGGCTTCTACGGGGCCTTCTACAGTGACGGGGAGATC
AGCATTTGCATGGAACACATGGACGGCGGCTCCCTGGACCAGGTGCTG
AAAGAGGCCAAGAGGATTCCCGAGGAGATCCTGGGGAAAGTCAGCATC
GCGGTTCTCCGGGGCTTGGCGTACCTCCGAGAGAAGCACCAGATCATG
CACCGAGATGTGAAGCCCTCCAACATCCTCGTGAACTCTAGAGGGGAG
ATCAAGCTGTGTGACTTCGGGGTGAGCGGCCAGCTCATAGACTCCATG
GCCAACTCCTTCGTGGGCACGCGCTCCTACATGGCTCCGGAGCGGTTG
CAGGGCACACATTACTCGGTGCAGTCGGACATCTGGAGCATGGGCCTG
TCCCTGGTGGAGCTGGCCGTCGGAAGGTACCCCATCCCCCCGCCCGAC
GCCAAAGAGCTGGAGGCCATCTTTGGCCGGCCCGTGGTCGACGGGGAA
GAAGGAGAGCCTCACAGCATCTCGCCTCGGCCGAGGCCCCCCGGGCGC
CCCGTCAGCGGTCACGGGATGGATAGCCGGCCTGCCATGGCCATCTTT
GAACTCCTGGACTATATTGTGAACGAGCCACCTCCTAAGCTGCCCAAC
GGTGTGTTCACCCCCGACTTCCAGGAGTTTGTCAATAAATGCCTCATC
AAGAACCCAGCGGAGCGGGCGGACCTGAAGATGCTCACAAACCACACC
TTCATCAAGCGGTCCGAGGTGGAAGAAGTGGATTTTGCCGGCTGGTTG
TGTAAAACCCTGCGGCTGAACCAGCCCGGCACACCCACGCGCACCGCC
GTGCACCATCACCATCACCATtaagaattcgc