*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

**Calculated molecular mass:-**
Monoisotopic 71, 894.00 daltons
Average Mass 71, 939.95 daltons
[cysteines reduced, methionines have not been oxidised

**Theoretical pI:-** 6.15

**Purity:-** >80 %

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

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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<br>GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE<br>GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN<br>GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY<br>LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**LARRKPVLP**<br>**ALTINPTIAEGPSPTSEGASEANLVDLQKKLEELELDEQQKKRLEAFL**<br>**TQKAKVGELKDDDFERISELGAGNGGVVTKVQHRPSGLIMARKLIHLE**<br>**IKPAIRNQIIRELQVLHECNSPYIVGFYGAFYSDGEISICMEHMDGGS**<br>**LDQVLKEAKRIPEEILGKVSIAVLRGLAYLREKHQIMHRDVKPSNILV**<br>**NSRGEIKLCDFGVSGQLIDSMANSFVGTRSYMAPERLQGTHYSVQSDI**<br>**WSMGLSLVELAVGRYPIPPPDAKELEAIFGRPVVDGEEGEPHSISPRP**<br>**RPPGRPVSGHGMDSRPAMAIFELLDYIVNEPPPKLPNGVFTPDFQEFV**<br>**NKCLIKNPAERADLKMLTNHTFIKRSEVEEVDFAGWLCKTLRLNQPGT**<br>**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