

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of MKK1 D208A [2 – 393]

<u>Enzyme description:-</u>	MKK1 D208A [2 - 393]
<u>Clone number:-</u>	DU 15938
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	<i>E.coli</i>
<u>Tag:-</u>	N-terminal GST and C-terminal His6
<u>Purification method:-</u>	GSH Sepharose

Calculated molecular mass:-

Monoisotopic 70, 865.24 daltons
Average Mass 70, 910.82 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.26

Purity:- >80 %

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, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

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Clone Data Sheet

MKK1 D208A [2 – 393]

<u>Protein</u>	MKK1 D208A [2 - 393]
<u>Clone number</u>	DU 15938
<u>Species</u>	Human
<u>Accession number</u>	L05624
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA VLDIRYGVSR IAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFFQGPLGSPKKKPTPIQLNPAP DGSAVNGTSSAETNLEALQKKLEELDEQQRKRLEAFLTQKQKVGELK DDDFEKISELGAGNGGVVFKVSHKPSGLVMARKLIHLEIKPAIRNQIIR ELQVLHECNSPYIVGFYGA FYSDGEISICMEHMDGGSLDQVLKKAGRIP EQILGKVSIAVIKGLTYLREKHKIMHRDVKPSNILVNSRGEIKLCAFGV SGQLIDSMANSFVGTRSYMSPERLQGTHYSVQSDIWSMGLSLVEMAVGR YPIPPDAKELELMFGCQVEGDAAETPPRPRTPGRPLSSYGMDSRPPMA IFELLDYIVNEPPPKLPSGVFSLEFQDFVNKCLIKNPAERADLKQLMVH AFIKRSDAEVDFAGWLCSTIGLNQ PSTPTHAAGVHHHHHH
<u>Native sequence</u>	Amino acids P2 – V393 (end) of human MKK1. 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 His6 tag is located at residues 624 – 629. The enzyme has a D207A mutation. Residue D208 is equivalent to A438 of the fusion protein.
<u>Protease cleavage</u>	Prescission site (<u>LEVLFQGP</u>) at residues 221 - 228
<u>Cloning sites</u>	<i>Bam</i> H1 site of pGEX-6P1

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Nucleotide Sequence of insert

ggatccCCCAAGAAGAAGCCGACGCCCATCCAGCTGAACCCGGCCCCCGA
CGGCTCTGCAGTTAACGGGACCAGCTCTGCGGAGACCAACTTGGAGGCCT
TGCAGAAGAAGCTGGAGGAGCTAGAGCTTGATGAGCAGCAGCGAAAGCGC
CTTGAGGCCTTTCTTACCCAGAAGCAGAAGGTGGGAGAAGTGAAGGATGA
CGACTTTGAGAAGATCAGTGAGCTGGGGGCTGGCAATGGCGGTGTGGTGT
TCAAGGTCTCCCACAAGCCTTCTGGCCTGGTCATGGCCAGAAAGCTAATT
CATCTGGAGATCAAACCCGCAATCCGGAACCAGATCATAAGGGAGCTGCA
GGTTCTGCATGAGTGCAACTCTCCGTACATCGTGGGCTTCTATGGTGCGT
TCTACAGCGATGGCGAGATCAGTATCTGCATGGAGCACATGGATGGAGGT
TCTCTGGATCAAGTCCTGAAGAAAGCTGGAAGAATTCCTGAACAAATTTT
AGGAAAAGTTAGCATTGCTGTAATAAAAGGCCCTGACATATCTGAGGGAGA
AGCACAAGATCATGCACAGAGATGTCAAGCCCTCCAACATCCTAGTCAAC
TCCCGTGGGGAGATCAAGCTCTGTGCCCTTTGGGGTCAGCGGGCAGCTCAT
CGACTCCATGGCCAACTCCTTCGTGGGCACAAGGTCTACATGTCGCCAG
AAAGACTCCAGGGGACTCATTACTCTGTGCAGTCAGACATCTGGAGCATG
GGACTGTCTCTGGTAGAGATGGCGGTTGGGAGGTATCCCATCCCTCCTCC
AGATGCCAAGGAGCTGGAGCTGATGTTTGGGTGCCAGGTGGAAGGAGATG
CGGCTGAGACCCACCCAGGCCAAGGACCCCGGGAGGCCCTTAGCTCA
TACGGAATGGACAGCCGACCTCCCATGGCAATTTTTGAGTTGTTGGATTA
CATAGTCAACGAGCCTCCTCCAAAAGTGGCCAGTGGAGTGTTCAGTCTGG
AATTTCAAGATTTTGTGAATAAATGCTTAATAAAAAACCCGCGAGAGAGA
GCAGATTTGAAGCAACTCATGGTTCATGCTTTTATCAAGAGATCTGATGC
TGAGGAAGTGGATTTTGCAGGTGGCTCTGCTCCACCATCGGCCTTAACC
AGCCCAGCACACCAACCCATGCTGCTGGCGTCCATCATCACCATCACCAT
tag