

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active MKK7 beta [2 - 419]

<u>Enzyme description:-</u>	MKK7 beta [2 - 419]
<u>Clone number:-</u>	DU 703
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	<i>E.coli</i>
<u>Tag:-</u>	N-terminal GST and FLAG
<u>Purification method:-</u>	GSH Sepharose
<u>Expression level:-</u>	>50 mg/L
<u>Calculated molecular mass:-</u>	74, 861 daltons
<u>Purity:-</u>	>70 %

Activation protocol:-

MKK7 beta (2.5 μ M) is activated in 50 mM Tris-HCl pH 7.5, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 10 mM MgAc, 0.1mM ATP, with 100 μ g/ml His-MEKK1 [DU 1847] at 30 °C for 30 min. Following activation, GST-MKK7 beta is re-purified by GSH Sepharose chromatography.

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270 mM sucrose, 150 mM NaCl, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.03 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

Assay:-

Two step assay in which MKK7 beta activates inactive JNK 1, 2 or 3 [DU 700, DU 699 or DU 1511], which then phosphorylates ATF2 [DU 1787].

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

MKK7 beta [2 - 419]

<u>Protein</u>	MKK7 beta [2 - 419]
<u>Clone number</u>	DU 703
<u>Species</u>	Human
<u>Accession number</u>	NM_145185
<u>Tags</u>	N-terminal GST and Flag
<u>Bacterially expressed protein</u>	MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNPLYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLHMFEDRLCHKTYLN GDHVTHPDFMLYDALAVVLYMDPMCLDAFPKLVCFKKRIEAIQIDKY LKSSKYIAWPLQGQWATFGGGDHPPKSDLVPRGSATMDYKDDDDKAAS SLEQKLSRLEAKLKQENREARRRIDLNLDISPQRPRPTLQLPLANDGG SRSPSESSPQHPTPPARPRHMLGLPSTLFTPRSMESIEIDQKLQEIM KQTGYLTIGGQRYQAEINDLENLGEMSGTCGQVWKMRFRKTGHVIAV KQMRRSGNKEENKRILMDLDVVLKSHDCPYIVQCFGTFITNTDVFIAV ELMGTCAEKLKKRMQGPPIPERILGKMTVAIVKALYYLKEKHGVIHRDV KPSNILLDERGQIKLCDFGISGRLVDSKAKTRSAGCAAYMAPERIDPP DPTKPDYDIRADVWSLGLISLVELATGQFPYKNCKTDFEVLTKVLQEEP PLLPGHMGFSGDFQSFVKDCLTKDHRKRPKYNKLEHSFIKRYETLEV DVASWFKDVMAKTESPRTSGVLSQPHLPFFR
<u>Native sequence</u>	Amino acids A2 – R419 (end) of human MKK7 beta. Residue A238 of the fusion protein is equivalent to A2 of the native enzyme. The GST tag is located at residues 1 – 220 and the Flag tag is located at residues 230 and 237.
<u>Protease cleavage</u>	Thrombin (<u>LVPRGS</u>) at residues 221 – 226
<u>Cloning sites</u>	<i>Bam</i> HI and <i>Xho</i> I sites of pGEX-4T-1

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Nucleotide sequence of insert

ATGGACTACAAGGACGACGATGACAAGGCGGCGTCCTCCCTGGAACAG
AAGCTGTCCCGCCTGGAAGCAAAGCTGAAGCAGGAGAACC GGGAGGCC
CGGCGGAGGATCGACCTCAACCTGGATATCAGCCCCCAGCGGCCAGG
CCCACCCTGCAGCTCCCGCTGGCCAACGATGGGGGCAGCCGCTCGCCA
TCCTCAGAGAGCTCCCCGCAGCACCCACGCCCCCGCCGGCCCCGC
CACATGCTGGGGCTCCCGTCAACCCTGTTACACCCCGCAGCATGGAG
AGCATTGAGATTGACCAGAAGCTGCAGGAGATCATGAAGCAGACGGGC
TACCTGACCATCGGGGGCCAGCGCTACCAGGCAGAAATCAACGACCTG
GAGAACTTGGGCGAGATGGGCAGCGGCACCTGCGGCCAGGTGTGGAAG
ATGCGCTTCCGGAAGACCGGCCACGTCAATTGCCGTTAAGCAAATGCGG
CGCTCCGGGAACAAGGAGGAGAACAAGCGCATCCTCATGGACCTGGAT
GTGGTGCTGAAGAGCCACGACTGCCCTACATCGTGCAGTGCTTTGGG
ACGTTTCATCACCAACACGGACGTCTTCATCGCCATGGAGCTCATGGGC
ACCTGCGCTGAGAAGCTCAAGAAGCGGATGCAGGGCCCCATCCCCGAG
CGCATTCTGGGCAAGATGACAGTGGCGATTGTGAAGGCGCTGTACTAC
CTGAAGGAGAAGCACGGTGTATCCACCGCGACGTCAAGCCCTCCAAC
ATCCTGCTGGACGAGCGGGGCCAGATCAAGCTCTGCGACTTCGGCATC
AGCGGCCGCCTGGTGGACTCCAAAGCCAAGACGCGGAGCGCCGGCTGT
GCCGCCTACATGGCACCCGAGCGCATTGACCCCCCAGACCCACCAAG
CCGGACTATGACATCCGGGGCCGACGTATGGAGCCTGGGCATCTCGTTG
GTGGAGCTGGCAACAGGACAGTTTCCCTACAAGAACTGCAAGACGGAC
TTTGAGGTCCTCACCAAAGTCCTACAGGAAGAGCCCCCGCTTCTGCCC
GGACACATGGGCTTCTCGGGGGACTTCCAGTCCTTCGTCAAAGACTGC
CTTACTAAAGATCACAGGAAGAGACCAAAGTATAATAAGCTACTTGAA
CACAGCTTCATCAAGCGCTACGAGACGCTGGAGGTGGACGTGGCGTCC
TGGTTCAAGGATGTCATGGCGAAGACTGAGTCACCGCGGACTAGCGGC
GTCCTGAGCCAGCCCCACCTGCCCTTCTTCAGGtag