

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active MKK6 [2 - 334]

<u>Enzyme description:-</u>	MKK6 [2 - 334]
<u>Clone number:-</u>	DU 1671
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	<i>E.coli</i>
<u>Tag:-</u>	N-terminal MBP (maltose binding protein)
<u>Purification method:-</u>	Amylose agarose
<u>Expression level:-</u>	5 mg/L
<u>Calculated molecular mass:-</u>	82, 970 daltons
<u>Purity:-</u>	>80 %

Activation protocol:-

MKK6 (2 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, MKK6 is re-purified by amylose agarose 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, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

Assay:-

Two step assay in which MKK6 activates inactive SAPK2a [DU 979], which then phosphorylates MBP.

Assay buffer:-

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

Specific activity range:- 500 – 1000 U/mg

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Clone Data Sheet

MKK6 [2 - 334]

Protein MKK6 [2 - 334]

Clone number DU 1671

Species Human

Accession number NM_002758

Tags N-terminal MBP

Bacterially expressed protein

MKIKTGARILALSALTTMMFSASALAKIEEGKLVIWINGDKGYNGLAE
VGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGPDIIFWAHDRFGGY
AQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYN
KDLLPNPPKTWEEIPALDKELKAKGKSALMFNLQEPYFTWPLIAADGG
YAFKYENGKYDIKDVGVNAGAKAGLTFLVDLIKNKHMNADTDYSIAE
AAFNKGETAMTINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGV
SAGINAASPNKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYEEE
LAKDPRIAATMENAQKGEIMPNIQMSAFWYAVRTAVINAASGRQTVD
EALKDAQTNSSSSNNNNNNNNNNNLGIEGRISEFGSS**R**SKGKKRNPGLKI
PKEAFEQPQTSSTPPRDLDSKACISIGNQNFEVKADDLEPIMELGRGA
YGVVEKMRHVPSPGQIMAVKRIRATVNSQEQKRLLMDLDISMRTVDCPF
TVTFYGFALFREGDVWICMELMDTSLDKFYKQVIDKGQTIPEDILGKIA
VSIVKALEHLHSLKLSVIHRDVKPSNVLINALGQVKMCDFGISGYLVDS
VAKTIDAGCKPYMAPERINPELNQKGYSVKSDIWSLGITMIELAILRF
PYDSWGTPFQQLKQVVEEPSQQLPADKFSAEFVDFTSQCLKKNSKERP
TYPELMQHPFFTLHESKGTDVASFVKLILGD

Native sequence Amino acids S2 – D334 (end) of human MKK6.
Residue S419 of the fusion protein is S2 of the native enzyme. The
MBP tag is located at residues 1 – 408.

The following amino acid substitution is present:
Q – **R**, where Q3 of the native enzyme is **R420** of the fusion protein.

Protease cleavage Factor Xa (**IEGR**) at residues 409 - 412

Cloning sites *Bam*H1 and *Hind*III site of pMAL

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Nucleotide
sequence of insert

GGATCCTCTAGATCGAAAGGCAAGAAGCGAAACCCTGGCCTTAAAATT
CCAAAAGAAGCATTTGAACAACCTCAGACCAGTTCCACACCACCTCGA
GATTTAGACTCCAAGGCTTGCATTTCTATTGGAAATCAGAACTTTGAG
GTGAAGGCAGATGACCTGGAGCCTATAATGGAACCTGGGACGAGGTGCG
TACGGGGTGGTGGAGAAGATGCGGCACGTGCCAGCGGGCAGATCATG
GCAGTGAAGCGGATCCGAGCCACAGTAAATAGCCAGGAACAGAAACGG
CTACTGATGGATTTGGATATTTCCATGAGGACGGTGGACTGTCCATTC
ACTGTCACCTTTTATGGCGCACTGTTTTCGGGAGGGTGATGTGTGGATC
TGCATGGAGCTCATGGATACATCACTAGATAAATTCTACAAACAAGTT
ATTGATAAAGGCCAGACAATTCCAGAGGACATCTTAGGGAAAATAGCA
GTTTCTATTGTAAAAGCATTAGAACATTTACATAGTAAGCTGTCTGTC
ATTCACAGAGACGTCAAGCCTTCTAATGTACTCATCAATGCTCTCGGT
CAAGTGAAGATGTGCGATTTTGAATCAGTGGCTACTTGGTGGACTCT
GTTGCTAAAACAATTGATGCAGGTTGCAAACCATACATGGCCCCTGAA
AGAATAAACCCAGAGCTCAACCAGAAGGGATACAGTGTGAAGTCTGAC
ATTTGGAGTCTGGGCATCACGATGATTGAGTTGGCCATCCTTCGATTT
CCCTATGATTCATGGGGAACCTCATTTCAGCAGCTCAAACAGGTGGTA
GAGGAGCCATCGCCACAACCTCCAGCAGACAAGTTCTCTGCAGAGTTT
GTTGACTTTACCTCACAGTGCTTAAAGAAGAATTCCAAAGAACGGCCT
ACATACCCAGAGCTAATGCAACATCCATTTTTTACCCTACATGAATCC
AAAGGAACAGATGTGGCATCTTTTGTAACACTGATTCTTGGAGACTaa
aagctt