

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active MKK6 [2 - 334] S207E T211D

<u>Enzyme description:-</u>	MKK6 [2 - 334] S207E T211D
<u>Clone number:-</u>	DU 495
<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>Calculated molecular mass:-</u>	
Monoisotopic	83, 026.45 daltons
Average Mass	83, 079.00 daltons
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	5.74
<u>Purity:-</u>	>80 %
<u>Activation protocol:-</u>	Constitutively active
<u>Enzyme storage buffer:-</u>	
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	
<u>Storage temperature:-</u>	-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

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Clone Data Sheet

MKK6 [2 - 334] S207E T211D

Protein MKK6 [2 - 334] S207E T211D

Clone number DU 495

Species Human

Accession number NM_002758

Tags N-terminal MBP

**Bacterially
expressed protein**

MKIKTGARILALSALTMMFSASALAKIEEGKLVIWINGDKGYNGLAE
VGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGPDIIFWAHDRFGGY
AQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYN
KDLLPNPPKTWEEIPALDKELKAKGKSALMFNLQEPYFTWPLIAADGG
YAFKYENGKYDIKDVGVNAGAKAGLTFLVDLIKNKHMNADTDYSIAE
AAFNKGETAMTINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGV
SAGINAASPNKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYYYY
LAKDPRIAATMENAQKGEIMPNIPOMSAFWYAVRTAVINAASGRQTV
EALKDAQTNSSNNNNNNNNNNNLGIEGRISEFGSS**RSKGKKRN**PGLKI
PKEAFEQPQTSSTPPRDLSKACISIGNQNFEVKADDLEPIMELGRGA
YGVVEKMRHVPSGQIMAVKRIRATVNS**QEQKRL**MDLDISMRTVDCPF
TVTFYGALFREGDVWICMELMDTSLDKFYKQVIDKGQTIPELILGKIA
VSIVKALEHLHSLSVIHRDVKPSNVLINALGQVKMCDFGISGYLVD**E**
VAKDIDAGCKPYMAPERINPELNQKGYSVKSDIWSLGITMIELAILRF
PYDSWGTPFQQLKQVVEEPSPQLPADKFSAEFVDFTSQCLKKNSKERP
TYPELMQHPFFTLHESKGTDVASFVKLILGD

Native sequence Amino acids S2 – D334 (end) of human MKK6.
Residue S419 of the fusion protein is S2 of the native enzyme. The enzyme has an S207**E** and T211**D** mutation to mimic phosphorylation of the enzyme. Residue S207 is equivalent to **E624** of the fusion protein and residue T211 is equivalent to **D628** of the fusion protein. 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

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Cloning sites

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

Nucleotide sequence of insert

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ggatccTCTAGATCGAAAGGCAAGAAGCGAAACCCTGGCCTTAAAATT  
CCAAAAGAAGCATTTGAACAACCTCAGACCAGTTCCACACCACCTCGA  
GATTTAGACTCCAAGGCTTGCATTTCTATTGGAAATCAGAACTTTGAG  
GTGAAGGCAGATGACCTGGAGCCTATAATGGAAC TGGGACGAGGTGCG  
TACGGGGTGGTGGAGAAGATGCGGCACGTGCCCAGCGGGCAGATCATG  
GCAGTGAAGCGGATCCGAGCCACAGTAAATAGCCAGGAACAGAAACGG  
CTACTGATGGATTTGGATATTTCCATGAGGACGGTGGACTGTCCATTC  
ACTGTCACCTTTTATGGCGCACTGTTTCGGGAGGGTGATGTGTGGATC  
TGCATGGAGCTCATGGATACATCACTAGATAAAATCTACAAACAAGTT  
ATTGATAAAGGCCAGACAATTCCAGAGGACATCTTAGGGAAAATAGCA  
GTTTCTATTGTAAAAGCATTAGAACATTTACATAGTAAGCTGTCTGTC  
ATTCACAGAGACGTCAAGCCTTCTAATGTACTCATCAATGCTCTCGGT  
CAAGTGAAGATGTGCGATTTTGGAATCAGTGGCTACTTGGTGGACGAA  
GTTGCTAAAGATATTGATGCAGGTTGCAAACCATACATGGCCCCTGAA  
AGAATAAACCCAGAGCTCAACCAGAAGGGATACAGTGTGAAGTCTGAC  
ATTTGGAGTCTGGGCATCACGATGATTGAGTTGGCCATCCTTCGATTT  
CCCTATGATTCATGGGGAAC TCCATTT CAGCAGCTCAAACAGGTGGTA  
GAGGAGCCATCGCCACAAC TCCAGCAGACAAGTTCTCTGCAGAGTTT  
GTTGACTTTACCTCACAGTGCTTAAAGAAGAATTCCAAAGAACGGCCT  
ACATACCCAGAGCTAATGCAACATCCATTTTTTCACCCTACATGAATCC  
AAAGGAACAGATGTGGCATCTTTTGTAAAAC T GATTCTTGGAGACTaa  
aagctt
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