Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active MKK6 [2 - 334] S207D T211E

Enzyme description:- MKK6 [2 - 334] S207D T211E

Clone number:- DU 594

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal MBP (maltose binding protein)

Purification method:- Amylose agarose

Calculated molecular mass:-
Monoisotopic 83, 026.45 daltons
Average Mass 83, 079.00 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.74

Purity:- >80 %

Activation protocol:- Constititively active

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 unactive 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] S207D T211E

Protein MKK6 [2 - 334] S207D T211E

Clone number DU 594

Species Human

Accession number NM_002758

Tags N-terminal MBP

Bacterially expressed protein

MKIKTGARILALSALTTMMFSASALAKIEEGKLVIWINGDKGYNGLAE
VGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGPDIIFWAHDRFGGY
AQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYN
KDLLPNPPKTWEEIPALDKELKAKGKSALMFNLQEPYFTWPLIAADGG
YAFKYENGKYDIKDVGVDNAGAKAGLTFLVDLIKNKHMNADTDYSIAE
AAFNKGETAMTINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVL
SAGINAASPNKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYEEE
LAKDPRIAATMENAQKGEIMPNIPQMSAFWYAVRTAVINAASGRQTVD
EALKDAQTNSSSNNNNNNNNNLGIEGRISEFGS**SRSKGKKRNPGLKI**
PKEAFEQPQTSSTPPRDLDSKACISIGNQNFEVKADDLEPIMELGRGA
YGVVEKMRHVPSGQIMAVKRIRATVNSQEQKRLLMDLDISMRTVDCPF
TVTFYGALFREGDVWICMELMDTSLDKFYKQVIDKGQTIPEDILGKIA
VSIVKALEHLHSKLSVIHRDVKPSNVLINALGQVKMCDFGISGYLVDD
VAKEIDAGCKPYMAPERINPELNQKGYSVKSDIWSLGITMIELAILRF
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 S207D and T211E mutation to mimic phosphorylation of the enzymes. Residue S207 is equivalent to D624 of the fusion protein and residue T211 is equivalent to E628 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

Cloning sites

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

Nucleotide sequence of insert

ggatccTCTAGATCGAAAGGCAAGAAGCGAAACCCTGGCCTTAAAATT
CCAAAAGAAGCATTTGAACAACCTCAGACCAGTTCCACACCACCTCGA
GATTTAGACTCCAAGGCTTGCATTTCTATTGGAAATCAGAACTTTGAG
GTGAAGGCAGATGACCTGGAGCCTATAATGGAACTGGGACGAGGTGCG
TACGGGGTGGTGGAGAAGATGCGGCACGTGCCCAGCGGGCAGATCATG
GCAGTGAAGCGGATCCGAGCCACAGTAAATAGCCAGGAACAGAAACGG
CTACTGATGGATTTGGATATTTCCATGAGGACGGTGGACTGTCCATTC
ACTGTCACCTTTTATGGCGCACTGTTTCGGGAGGGTGATGTGTGGATC
TGCATGGAGCTCATGGATACATCACTAGATAAATTCTACAAACAAGTT
ATTGATAAAGGCCAGACAATTCCAGAGGACATCTTAGGGAAAATAGCA
GTTTCTATTGTAAAAGCATTAGAACATTTACATAGTAAGCTGTCTGTC
ATTCACAGAGACGTCAAGCCTTCTAATGTACTCATCAATGCTCTCGGT
CAAGTGAAGATGTGCGATTTTGGAATCAGTGGCTACTTGGTGGACGAT
GTTGCTAAAGAAATTGATGCAGGTTGCAAACCATACATGGCCCCTGAA
AGAATAAACCCAGAGCTCAACCAGAAGGGATACAGTGTGAAGTCTGAC
ATTTGGAGTCTGGGCATCACGATGATTGAGTTGGCCATCCTTCGATTT
CCCTATGATTCATGGGGAACTCCATTTCAGCAGCTCAAACAGGTGGTA
GAGGAGCCATCGCCACAACTCCCAGCAGACAAGTTCTCTGCAGAGTTT
GTTGACTTTACCTCACAGTGCTTAAAGAAGAATTCCAAAGAACGGCCT
ACATACCCAGAGCTAATGCAACATCCATTTTTCACCCTACATGAATCC
AAAGGAACAGATGTGGCATCTTTTGTAAAACTGATTCTTGGAGACtaa
aagctt