Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active MKK6 [2 - 334]

Enzyme description:- MKK6 [2 - 334]

Clone number:- DU 1671

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal MBP

Purification method:- Amylose agarose

Calculated molecular mass:-

Monoisotopic 82, 970.46 daltons
Average Mass 83, 022.98 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.92

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

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]

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
YAFKYENGKYDIKDVGVDNAGAKAGLTFLVDLIKNKHMNADTDYSIAE
AAFNKGETAMTINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVL
SAGINAASPNKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYEEE
LAKDPRIAATMENAQKGEIMPNIPQMSAFWYAVRTAVINAASGRQTVD
EALKDAQTNSSSNNNNNNNNNLGIEGRISEFGS**SRSKGKKRNPGLKI**
PKEAFEQPQTSSTPPRDLDSKACISIGNQNFEVKADDLEPIMELGRGA
YGVVEKMRHVPSGQIMAVKRIRATVNSQEQKRLLMDLDISMRTVDCPF
TVTFYGALFREGDVWICMELMDTSLDKFYKQVIDKGQTIPEDILGKIA
VSIVKALEHLHSKLSVIHRDVKPSNVLINALGQVKMCDFGISGYLVDS
VAKTIDAGCKPYMAPERINPELNQKGYSVKSDIWSLGITMIELAILRF
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 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
CAAGTGAAGATGTGCGATTTTGGAATCAGTGGCTACTTGGTGGACTCT
GTTGCTAAAACAATTGATGCAGGTTGCAAACCATACATGGCCCCTGAA
AGAATAAACCCAGAGCTCAACCAGAAGGGATACAGTGTGAAGTCTGAC
ATTTGGAGTCTGGGCATCACGATGATTGAGTTGGCCATCCTTCGATTT
CCCTATGATTCATGGGGAACTCCATTTCAGCAGCTCAAACAGGTGGTA
GAGGAGCCATCGCCACAACTCCCAGCAGACAAGTTCTCTGCAGAGTTT
GTTGACTTTACCTCACAGTGCTTAAAGAAGAATTCCAAAGAACGGCCT
ACATACCCAGAGCTAATGCAACATCCATTTTTCACCCTACATGAATCC
AAAGGAACAGATGTGGCATCTTTTGTAAAACTGATTCTTGGAGACtaa
aagctt