Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of MKK6 [1 - 334] K82A

Enzyme description:- MKK6 [1 - 334] K82A

Clone number:- DU 50032

Source:- Recombinant

Expression system:- *E.coli*

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

Purification method:- Amylose agarose

Calculated molecular mass:-
Monoisotopic 83, 038.24 daltons
Average Mass 83, 090.83 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.27

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

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Clone Data Sheet

MKK6 [1 - 334] K82A

Protein MKK6 [1 - 334] K82A

Clone number DU 50032

Species Human

Accession number NM_002758

Tags N-terminal MBP

Bacterially expressed protein

MMKIEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKF
PQVAATGDGPDIIFWAHDRFGGYAQSGLLAEITPDKAFQDKLYPFTWDA
VRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPALDKELKAKGKS
ALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIKDVGVDNAGAKAGLTF
LVDLIKNKHMNADTDYSIAEAAFNKGETAMTINGPWAWSNIDTSKVNYG
VTVLPTFKGQPSKPFVGVLSAGINAASPNKELAKEFLENYLLTDEGLEA
VNKDKPLGAVALKSYEEELVKDPRIAATMENAQKGEIMPNIPQMSAFWY
AVRTAVINAASGRQTVDEALKDAQTNSSSNNNNNNNNNNLGDDDDKVPE
FLEVLFQGPLGSPGEFASLEKLAAA**MSQSKGKKRNPGLKIPKEAFEQPQ
TSSTPPRDLDSKACISIGNQNFEVKADDLEPIMELGRGAYGVVEKMRHV
PSGQIMAV**A**RIRATVNSQEQKRLLMDLDISMRTVDCPFTVTFYGALFRE
GDVWICMELMDTSLDKFYKQVIDKGQTIPEDILGKIAVSIVKALEHLHS
KLSVIHRDVKPSNVLINALGQVKMCDFGISGYLVDSVAKTIDAGCKPYM
APERINPELNQKGYSVKSDIWSLGITMIELAILRFPYDSWGTPFQQLKQ
VVEEPSPQLPADKFSAEFVDFTSQCLKKNSKERPTYPELMQHPFFTLHE
SKGTDVASFVKLILGD**

Native sequence Amino acids M1 – D334 (end) of human MKK6.
Residue M418 of the fusion protein is M1 of the native enzyme. The enzyme has a K82A mutation to produce a kinase dead enzyme. Residue K82 is equivalent to A499 of the fusion protein. The MBP tag is located at residues 1 – 393.

Protease cleavage PreScission (LEVLFQGP) residues 394 - 401

Cloning sites

*Not*1 sites of pMEX3Cb

Nucleotide sequence of insert

gcggccgcgATGTCTCAGTCGAAAGGCAAGAAGCGAAACCCTGGCCTT
AAAATTCCAAAAGAAGCATTTGAACAACCTCAGACCAGTTCCACACCA
CCTCGAGATTTAGACTCCAAGGCTTGCATTTCTATTGGAAATCAGAAC
TTTGAGGTGAAGGCAGATGACCTGGAGCCTATAATGGAACTGGGACGA
GGTGCGTACGGGGTGGTGGAGAAGATGCGGCACGTGCCCAGCGGGCAG
ATCATGGCAGTGGCGCGGATCCGAGCCACAGTAAATAGCCAGGAACAG
AAACGGCTACTGATGGATTTGGATATTTCCATGAGGACGGTGGACTGT
CCATTCACTGTCACCTTTTATGGCGCACTGTTTCGGGAGGGTGATGTG
TGGATCTGCATGGAGCTCATGGATACATCACTAGATAAATTCTACAAA
CAAGTTATTGATAAAGGCCAGACAATTCCAGAGGACATCTTAGGGAAA
ATAGCAGTTTCTATTGTAAAAGCATTAGAACATTTACATAGTAAGCTG
TCTGTCATTCACAGAGACGTCAAGCCTTCTAATGTACTCATCAATGCT
CTCGGTCAAGTGAAGATGTGCGATTTTGGAATCAGTGGCTACTTGGTG
GACTCTGTTGCTAAAACAATTGATGCAGGTTGCAAACCATACATGGCC
CCTGAAAGAATAAACCCAGAGCTCAACCAGAAGGGATACAGTGTGAAG
TCTGACATTTGGAGTCTGGGCATCACGATGATTGAGTTGGCCATCCTT
CGATTTCCCTATGATTCATGGGGAACTCCATTTCAGCAGCTCAAACAG
GTGGTAGAGGAGCCATCGCCACAACTCCCAGCAGACAAGTTCTCTGCA
GAGTTTGTTGACTTTACCTCACAGTGCTTAAAGAAGAATTCCAAAGAA
CGGCCTACATACCCAGAGCTAATGCAACATCCATTTTTCACCCTACAT
GAATCCAAAGGAACAGATGTGGCATCTTTTGTAAAACTGATTCTTGGA
GACtaagcggccgc