

MRC PPU Reagents and Services

Standard Operating Procedure

Preparation of MEKK1 [1193 – 1512]

Enzyme description:- MEKK1 [1193 – 1512]

Clone number:- DU 68107

Source:- Recombinant

Expression system:- *E.coli*,

Tag:- N-terminal MBP

Purification method:- Amylose resin

Calculated molecular mass:-

Monoisotopic 79, 538.34 daltons

Average Mass 79, 588.33 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.35

Purity:- >80 %

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM sucrose, 2 mM MnCl₂,
0.1 % 2-mercaptoethanol, 0.03 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

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Clone Data Sheet

MEKK1 [1193 - 1512]

Protein MEKK1 [1193 - 1512]

Clone number DU 68107

Species Human

Accession number NM_005921.2

Tags N-terminal MBP

**Bacterially
expressed MEKK1
protein**

MKIEEGKLVIIWINGDKGYNGLAEVGGKFEKDTGIKVTVEHPDKLEEF
PQVAATGDGPDIIFWAHDRFGGYAQSGLLAEITPDKAFQDKLYPFTWD
AVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPALDKELKAKG
KSALMFNLQEPYFTWPLIAADGGYAFKYENGGYDIKDVGVNDAGAKAG
LTFVLVDLIKKNHMNADTDYSIAEAAFNKGETAMTINGPWAWSNIDTSK
VNYGVTVLPPTFKGQPSKPFVGVLSAGINAASPNKELAKEFLENYLLTD
EGLEAVNKDKPLGAVALKSYEEELVKDPRIAATMENAQGEIMPNIPO
MSAFWYAVRTAVINAASGRQTVDEALKDAQTNSSNNNNNNNNNNLGD
DDDKVPEFLEVLFGQPLGSMAMSASQDALPIVPQLQVENGEDI I I IQQ
DTPETLPGHTKAKQPYREDTEWLKGOQIGLGAFFSSCYQAQDVGTGTLM
AVKQVTYVRNTSSEQEEVVEALREEIRMSHLNHPNII RMLGATCEKS
NYNLFIEWMAGGSVAHLLSKYGAFKESVVINYTEQLLRGLSYLHENQI
IHRDVKGANLLIDSTGQRLRIADFGAAARLASKGTGAGEFQQLLGTI
AFMAPEVLRGQOYGRSCDVWSVGCAL IEMACAKPPWNAEKHSNHLALI
FKIASATTAPSI PSHLSPGLRDVALRCLELQPQDRPPSRELLKHPVFR
TTW

Native sequence Amino acids M1193 – W1512 (end) of human MEKK1.
Residue M404 of the fusion protein is equivalent to M1 of the native
enzyme. The MBP tag is located at residues 1 - 392.

Protease cleavage PreScission (LEVLFQGP) residues 393 - 400

Cloning sites *Bam*H1 and *Not*1 sites of pMEX3C

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Nucleotide Sequence Of Insert

ggatccATGGCAATGTCAGCGTCTCAGGATGCCCTCCCATAGTTCCT
CAGCTGCAGGTTGAAAATGGAGAAGATATCATCATTATTCAACAGGAT
ACACCAGAGACTCTACCAGGACATACCAAAGCAAACAACCGTATAGA
GAAGACACTGAATGGCTGAAAGGTCAACAGATAGGCCTTGGAGCATT
TCTTCTTGTATCAGGCTCAAGATGTGGGAAGTGAACCTTTAATGGCT
GTTAAACAGGTGACTTATGTCAGAAACACATCTTCTGAGCAAGAAGAA
GTAGTAGAAGCACTAAGAGAAGAGATAAGAATGATGAGCCATCTGAAT
CATCCAAACATCATTAGGATGTTGGGAGCCACGTGTGAGAAGAGCAAT
TACAATCTCTTCATTGAATGGATGGCAGGGGGATCGGTGGCTCATTG
CTGAGTAAATATGGAGCCTTCAAAGAATCAGTAGTTATTAACACTACT
GAACAGTTACTCCGTGGCCTTTCGTATCTCCATGAAAACCAAATCATT
CACAGAGATGTCAAAGGTGCCAATTTGCTAATTGACAGCACTGGTCAG
AGACTAAGAATTGCAGATTTTTGGAGCTGCAGCCAGGTTGGCATCAAAA
GGAAGTGGTGCAGGAGAGTTTTCAGGGACAATTACTGGGGACAATTGCA
TTTATGGCACCTGAGGTACTAAGAGGTCAACAGTATGGGAGGAGCTGT
GATGTATGGAGTGTGGCTGTGCTATTATAGAAATGGCTTGTGCAAAA
CCACCATGGAATGCAGAAAAACACTCCAATCATCTTGCTTTGATATTT
AAGATTGCTAGTGCAACTACTGCTCCATCGATCCCTTCACATTTGTCT
CCTGGTTTACGAGATGTGGCTCTTCGTTGTTTAGAACTTCAACCTCAG
GACAGACCTCCATCAAGAGAGCTACTGAAGCATCCAGTCTTTCGTA
ACATGGtaggcggccgc