

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active MELK [2 - 651]

<u>Enzyme description:-</u>	MELK [2 - 651]
<u>Clone number:-</u>	DU 1199
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	<i>E.coli</i>
<u>Tag:-</u>	N-terminal GST and C-terminal His(6)
<u>Purification method:-</u>	GSH Sepharose followed by Ni ²⁺ -NTA agarose
<u>Expression level:-</u>	0.5 mg/L
<u>Calculated molecular mass:-</u>	102, 092 daltons
<u>Purity:-</u>	90 %
<u>Activation protocol:-</u>	Constitutively active
<u>Enzyme storage buffer:-</u>	50 mM Tris-HCl pH 7.5, 270mM 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
<u>Assay:-</u>	Standard filter binding assay
<u>Assay buffer:-</u>	50 mM Tris-HCl pH 7.5, 0.1 % 2- mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc
<u>Substrate:-</u>	[KKLNRTLSEFAEPG] Final concentration: 200 µM
<u>Specific activity range:-</u>	150 – 300 U/mg

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Clone Data Sheet

MELK [2 - 651]

<u>Protein</u>	MELK [2 - 651]
<u>Clone number</u>	DU 1199
<u>Species</u>	Human
<u>Accession number</u>	NM_014791
<u>Tags</u>	N-terminal GST and C-terminal His(6)
<u>Bacterially expressed protein</u>	MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA VLDIRYGVSR IAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAI PQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVL FQG PLGSKDYDELLKYYELHE TIGTGGFAKVKLACHILTGEMVAIKIMDKNTLGSDLPRIKTEIEALKNL RHQHICQLYHVLETANKIFMVLEYCPGGELFDYIISQDRLSEEETRVVF RQIVSAVAYVHSQGYAHRDLKPENLLFDEYHKLKLIDFGLCAKPKGNKD YHLQTCCGSLAYAAPELIQGKSYLGSEADVWSMGILLYVLMCGFLPFDD DNVMALYKKIMRGKYDVPKWLSPSSILLQQLQVDPKKRISMKNLLNH PWIMQDYNYPVEWQSKNPF IHLDDDCVTELSVHHRNNRQT MEDLISLWQ YDHLTATYLLLLAKKARGKPVRLRLSSFSCGQASATPFTDIKSNNWSLE DVTASDKNYVAGLIDYDWCEDDLSTGAATPRTSQFTKYWTESNGVESKS LTPALCRTPANKLKNKENVYTPKSAVKNEEYFMFPEPKTPVNKNQHKRE ILTTPNRYTTPSKARNQCLKETPIKIPVNSTGTDKLMTGVISPERRCRS VELDLNQA HMEETPKRKGA KVFGLERGLDKVITVLTRSKRKGSARDGP RRLKLHYNVTTTRLVNP DQLLNEIMSILPKKHVDFVQGYTLKCQTQSD FGKVTMQFELEV CQLQKPDVVGIR RQLKGD AWVYKRLVEDILSSCKVH HHHHH
<u>Native sequence</u>	Amino acids K2 – V651 (end) of human MELK. Residue K232 of the fusion protein is equivalent to K2 of the native enzyme. The GST tag is located at residues 1 - 220 and the His(6) is located at residues 882 - 887
<u>Protease cleavage</u>	PreScission (<u>LEVLFQG PL</u>) residues 221 - 229
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> I site of pGEX 6P-1

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Nucleotide Sequence of insert

GGATCCAAAGATTATGATGAACTTCTCAAATATTATGAATTA
CATGAAACTATTGGGACAGGTGGCTTTGCAAAGGTCAAACCTT
GCCTGCCATATCCTTACTGGAGAGATGGTAGCTATAAAAATC
ATGGATAAAAACACACTAGGGAGTGATTTGCCCCGGATCAAA
ACGGAGATTGAGGCCTTGAAGAACCTGAGACATCAGCATATA
TGTCAACTCTACCATGTGCTAGAGACAGCCAACAAAATATTC
ATGGTTCTTGAGTACTGCCCTGGAGGAGAGCTGTTTGACTAT
ATAATTTCCCAGGATCGCCTGTCAGAAGAGGAGACCCGGGT
GTCTTCCGTCAGATAGTATCTGCTGTTGCTTATGTGCACAGC
CAGGGCTATGCTCACAGGGACCTCAAGCCAGAAAATTTGCTG
TTTGATGAATATCATAAATTAAGCTGATTGACTTTGGTCTC
TGTGCAAACCCAAGGGTAACAAGGATTACCATCTACAGACA
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CACAGAAACAACAGGCAAACAATGGAGGATTTAATTTCACTG
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AGGAAGGGTTCTGCCAGAGACGGGCCCAAGACTAAAGCTT
CACTATAATGTGACTACAACCTAGATTAGTGAATCCAGATCAA
CTGTTGAATGAAATAATGTCTATTCTTCCAAAGAAGCATGTT

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GACTTTGTACAAAAGGGTTATACACTGAAGTGTCAAACACAG
TCAGATTTTGGGAAAGTGACAATGCAATTTGAATTAGAAGTG
TGCCAGCTTCAAAAACCCGATGTGGTGGGTATCAGGAGGCAG
CGGCTTAAGGGCGATGCCTGGGTTTACAAAAGATTAGTGGAA
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taggcggccgc