

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 μ M
<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 LEFPNLPYYIDGDVKLTQSMATIRYIADKHNMLGGCPKERAETSMLEGA VLDIRYGVSRAYS KDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFGGPLGSKDYDELLKYYELHE TIGTGGFAKVKLACHILTGMVAIKIMDKNTLGSDLPRIKTEIEALKNL RHQHICQLYHVLETANKIFMVLEYCPGGELFDYIISQDRLSEEETRVVF RQIVSAVAYVHSQGYAHRDLKPENLLFDEYHKLKLIDFGLCAKPKGNKD YHLQTCCGSLAYAAPELIQGKSYLGSEADVWSMGILLYVLMCGFLPFDD DNVMALYKKIMRGKYDVPKWLS PSSILLQOMLQVDPKKRISMKNLLNH PWIMQDYNYPVEWQSKNPF IHLDDDCVTELSVHHRNNRQTMEDLISLWQ YDHLTATYLLLLAKKARGKPVRLRLSSFSCGQASATPFTDIKSNNWSLE DVTASDKNYVAGLIDYDWCEDDLSTGAATPRTSQFTKYWTESNGVESKS LTPALCRTPANKLKNKENVYTPKSAVKNEEYFMFPEPKTPVNKNQHKRE ILTTPNRYTTPSKARNQCLKETPIKIPVNSTGTDKLMTGVISPERRCRS VELDLNQAHEETPKRKGAKVFGSLERGLDKVITVLTRSKRKGSARDGP RRLKLHYNVTTTRLVNPQDLLNEIMSILPKKHVDFVQKGYTLKCQTQSD FGKVTMQFELEVCLQKPDVVGIRRQRLKGDWVYKRLVEDILSSCKVH 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>LEVLFQGPL</u>) residues 221 - 229
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> I site of pGEX 6P-1

Nucleotide
Sequence of insert

GGATCCAAAGATTATGATGAACTTCTCAAATATTATGAATTA
CATGAAACTATTGGGACAGGTGGCTTTGCAAAGGTCAAACCTT
GCCTGCCATATCCTTACTGGAGAGATGGTAGCTATAAAAATC
ATGGATAAAAACACACTAGGGAGTGATTGCCCCGGATCAAA
ACGGAGATTGAGGCCTTGAAGAACCTGAGACATCAGCATATA
TGTCAACTCTACCATGTGCTAGAGACAGCCAACAAAATATTC
ATGGTTCTTGAGTACTGCCCTGGAGGAGAGCTGTTTGACTAT
ATAATTTCCCAGGATCGCCTGTCAGAAGAGGAGACCCGGGT
GTCTTCCGTCAGATAGTATCTGCTGTTGCTTATGTGCACAGC
CAGGGCTATGCTCACAGGGACCTCAAGCCAGAAAATTTGCTG
TTTGATGAATATCATAAATTAAAGCTGATTGACTTTGGTCTC
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GATGATGATAATGTAATGGCTTTATACAAGAAGATTATGAGA
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CACAGAAACAACAGGCAAACAATGGAGGATTTAATTTCACTG
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GACTTTGTACAAAAGGGTTATACACTGAAGTGTCAAACACAG
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TGCCAGCTTCAAAAACCCGATGTGGTGGGTATCAGGAGGCAG
CGGCTTAAGGGCGATGCCTGGGTTTACAAAAGATTAGTGGAA
GACATCCTATCTAGCTGCAAGGTACACCATCACCATCACCAT
taggcggccgc