

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active smooth muscle MLCK [475 - 838]

Enzyme description:- smooth muscle MLCK [475 - 838]

Clone number:- DU 1106

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 3 mg/L

Calculated molecular mass:-

Monoisotopic 68, 727.67 daltons

Average Mass 68, 771.90 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.36

Purity:- 90 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 50 % glycerol, 150 mM NaCl, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -20 °C

Assay:- Standard filter binding assay

Assay buffer:-

50 mM Hepes-NaOH pH 7.5, 0.5 mM CaCl₂, 1 µM calmodulin,
0.1 % 2- mercaptoethanol, 10 mM magnesium acetate

Substrate:- KKRPPQRATSNVFA

Residues 12–23 (from second K) of human smooth muscle myosin regulatory light chain
Final concentration: 300 µM

Specific activity range:- 30 – 60 U/mg

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Clone Data Sheet

smooth muscle MLCK [475 - 838]

<u>Protein</u>	smooth muscle MLCK [475 - 838]
<u>Clone number</u>	DU 1106
<u>Species</u>	Human
<u>Accession number</u>	NM_005965
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA VLDIRYGVSR IAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAI PQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVL FQGPLGSDHEYKFRVRAIN VY GTSEPSQESELTTVG EKPEEPKDEVEVSDDDEKEPEVDYRTVTINTEQK VSDFYDIEERLGSGKFGQVFRLVEKKTRK V WAGKFFKAYS AKEKENIRQ EISIMNCLHHPKLVQCVD AFE EKANIVMVLEIVSGGELFERIIDEDFEL TERECIKYMRQISEGVEYIHKQGIVHLDLKPENIMCVNKTGTRIKLIDF GLARRLENAGSLKVLFGTPEFVAPEVINYEPIGYATDMWSIGVICYILV SGLSPFMGDNDNETLANVTSATWDFDDEAFDEISDDAKDFISNLLKKDM KNRLDCTQCLQHPWLMKDTKNMEAKKLSKDRMKKYMARRKWQKTGNAVR AIGRLSS
<u>Native sequence</u>	Amino acids D475 – S838 of human smooth muscle MLCK. [Full length protein ends at residue E991] Residue D232 of the fusion protein is equivalent to D2 of the native enzyme. The GST tag is located at residues 1 - 220
<u>Protease cleavage</u>	PreScission (<u>LEVLFQGPL</u>) residues 221 - 229
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Eco</i> R1 site of pGEX 6P-1

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Nucleotide

Sequence of insert

GGATCCGACCACGAATATAAGTTCCGTGTACGTGCAATCAAC
GTGTATGGAACCAGTGAGCCAAGCCAGGAGTCTGAACTCACA
ACGGTAGGAGAGAAACCTGAAGAGCCGAAGGATGAAGTGGAG
GTGTCAGATGATGATGAGAAGGAGCCCGAGGTTGATTACCGG
ACAGTGACAATCAATACTGAACAAAAAGTATCTGACTTCTAC
GACATTGAGGAGAGATTAGGATCTGGGAAATTTGGACAGGTC
TTTCGACTTGTAGAAAAGAAAACCTCGAAAAGTCTGGGCAGGG
AAGTTCTTCAAGGCATATTCAAGAAAAGAGAAAGAGAATATC
CGGCAGGAGATTAGCATCATGAACTGCCTCCACCACCCTAAG
CTGGTCCAGTGTGTGGATGCCTTTGAAGAAAAGGCCAACATC
GTCATGGTCCTGGAGATCGTGTGAGGAGGGGAGCTGTTTGAG
CGCATCATTGACGAGGACTTTGAGCTGACGGAGCGTGAGTGC
ATCAAGTACATGCGGCAGATCTCGGAGGGAGTGAGTACATC
CACAAGCAGGGCATCGTGCACCTGGACCTCAAGCCGGAGAAC
ATCATGTGTGTCAACAAGACGGGCACCAGGATCAAGCTCATC
GACTTTGGTCTGGCCAGGAGGCTGGAGAATGCGGGGTCTCTG
AAGGTCCTCTTTGGCACCCCAGAATTTGTGGCTCCTGAAGTG
ATCAACTATGAGCCCATCGGCTACGCCACAGACATGTGGAGC
ATCGGGGTCATCTGCTACATCCTAGTCAGTGGCCTTTCCCCC
TTCATGGGAGACAACGATAACGAAACCTTGGCCAACGTTACC
TCAGCCACCTGGGACTTCGACGACGAGGCATTTCGATGAGATC
TCCGACGATGCCAAGGATTTATCAGCAATCTGCTGAAGAAA
GATATGAAAAACCGCCTGGACTGCACGCAGTGCCTTCAGCAT
CCATGGCTAATGAAAGATACCAAGAACATGGAGGCCAAGAAA
CTCTCCAAGGACCGGATGAAGAAGTACATGGCAAGAAGGAAA
TGGCAGAAAACGGGCAATGCTGTGAGAGCCATTGGAAGACTG
TCCTCTtaggaattc