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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:- 68, 727 daltons

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, 5 mM $CaCl_2$, 10 μM calmodulin, 0.1 mM EGTA,
0.1 % 2–mercaptoethanol, 10 mM magnesium acetate

Substrate:-
KKRPQRATSNVFA
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]

Protein	smooth muscle MLCK [475 - 838]
Clone number	DU 1106
Species	Human
Accession number	NM_005965
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**DHEYKFRVRAINVY GTSEPSQESELTTVGEKPEEPKDEVEVSDDDEKEPEVDYRTVTINTEQK VSDFYDIEERLGSGKFGQVFRLVEKKTRKVWAGKFFKAYSAKEKENIRQ EISIMNCLHHPKLVQCVDAFEEKANIVMVLEIVSGGELFERIIDEDFEL TERECIKYMRQISEGVEYIHKQGIVHLDLKPENIMCVNKTGTRIKLIDF GLARRLENAGSLKVLFGTPEFVAPEVINYEPIGYATDMWSIGVICYILV SGLSPFMGDNDNETLANVTSATWDFDDEAFDEISDDAKDFISNLLKKDM KNRLDCTQCLQHPWLMKDTKNMEAKKLSKDRMKKYMARRKWQKTGNAVR AIGRLSS**
Native sequence	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
Protease cleavage	PreScission (LEVLFQGPL) residues 221 - 229
Cloning sites	*Bam*H1 and *Eco*R1 site of pGEX 6P-1

Nucleotide Sequence of insert

GGATCCGACCACGAATATAAGTTCCGTGTACGTGCAATCAAC
GTGTATGGAACCAGTGAGCCAAGCCAGGAGTCTGAACTCACA
ACGGTAGGAGAGAAACCTGAAGAGCCGAAGGATGAAGTGGAG
GTGTCAGATGATGATGAGAAGGAGCCCGAGGTTGATTACCGG
ACAGTGACAATCAATACTGAACAAAAAGTATCTGACTTCTAC
GACATTGAGGAGAGATTAGGATCTGGGAAATTTGGACAGGTC
TTTCGACTTGTAGAAAAGAAAACTCGAAAAGTCTGGGCAGGG
AAGTTCTTCAAGGCATATTCAGCAAAAGAGAAAGAGAATATC
CGGCAGGAGATTAGCATCATGAACTGCCTCCACCACCCTAAG
CTGGTCCAGTGTGTGGATGCCTTTGAAGAAAAGGCCAACATC
GTCATGGTCCTGGAGATCGTGTCAGGAGGGGAGCTGTTTGAG
CGCATCATTGACGAGGACTTTGAGCTGACGGAGCGTGAGTGC
ATCAAGTACATGCGGCAGATCTCGGAGGGAGTGGAGTACATC
CACAAGCAGGGCATCGTGCACCTGGACCTCAAGCCGGAGAAC
ATCATGTGTGTCAACAAGACGGGCACCAGGATCAAGCTCATC
GACTTTGGTCTGGCCAGGAGGCTGGAGAATGCGGGGTCTCTG
AAGGTCCTCTTTGGCACCCCAGAATTTGTGGCTCCTGAAGTG
ATCAACTATGAGCCCATCGGCTACGCCACAGACATGTGGAGC
ATCGGGGTCATCTGCTACATCCTAGTCAGTGGCCTTTCCCCC
TTCATGGGAGACAACGATAACGAAACCTTGGCCAACGTTACC
TCAGCCACCTGGGACTTCGACGACGAGGCATTCGATGAGATC
TCCGACGATGCCAAGGATTTCATCAGCAATCTGCTGAAGAAA
GATATGAAAAACCGCCTGGACTGCACGCAGTGCCTTCAGCAT
CCATGGCTAATGAAAGATACCAAGAACATGGAGGCCAAGAAA
CTCTCCAAGGACCGGATGAAGAAGTACATGGCAAGAAGGAAA
TGGCAGAAAACGGGCAATGCTGTGAGAGCCATTGGAAGACTG
TCCTCTtaggaattc