Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active MYLK2 [1 - 596]

Enzyme description:- MYLK2 [1 – 596]

Clone number:- DU 62899

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 92, 118.78 daltons
Average Mass 92, 177.70 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.23

Purity:- >80 %

Activation protocol:- Constitutively Active

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70 deg C

Assay buffer:-

50 mM HEPES-NaOH pH 7.5, 0.1 % 2 -mercaptoethanol, 10 mM magnesium acetate, 0.1 mM CaCl2, 10 µM Calmodulin

Substrate:-

KKRPQRATSNVFA Final concentration: 300 µM

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Clone Data Sheet

MYLK2 [1 – 596]

Protein	MYLK2 [1 – 596]
Clone number	DU 62899
Species	Human
Accession number	NM_033118.3
Tags	N-terminal GST
Baculovirus expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSPNSRVD**MAT** **ENGAVELGIQNPSTDKAPKGPTGERPLAAGKDPGPPDPKKAPDPPTLK** **KDAKAPASEKGDGTLAQPSTSSQGPKGEGDRGGGPAEGSAGPPAALPQ** **QTATPETSVKKPKAEQGASGSQDPGKPRVGKKAAEGQAAARRGSPAFL** **HSPSCPAIISSSEKLLAKKPPSEASELTFEGVPMTHSPTDPRPAKAEE** **GKNILAESQKEVGEKTPGQAGQAKMQGDTSRGIEFQAVPSEKSEVGQA** **LCLTAREEDCFQILDDCPPPPAPFPHRMVELRTGNVSSEFSMNSKEAL** **GGGKFGAVCTCMEKATGLKLAAKVIKKQTPKDKEMVLLEIEVMNQLNH** **RNLIQLYAAIETPHEIVLFMEYIEGGELFERIVDEDYHLTEVDTMVFV** **RQICDGILFMHKMRVLHLDLKPENILCVNTTGHLVKIIDFGLARRYNP** **NEKLKVNFGTPEFLSPEVVNYDQISDKTDMWSMGVITYMLLSGLSPFL** **GDDDTETLNNVLSGNWYFDEETFEAVSDEAKDFVSNLIVKDQRARMNA** **AQCLAHPWLNNLAEKAKRCNRRLKSQILLKKYLMKRRWKKNFIAVSAA** **NRFKKISSSGALMALGV**
Native sequence	Amino acids M1 – V596 (end) of human MYLK2. Residue M238 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	PreScission (LEVLFQGP) residues 221 - 228
Cloning sites	*Sal*1 - *Not*1 sites of pFastBac GST

Nucleotide sequence of insert

gtcgacATGGCGACAGAAAATGGAGCAGTTGAGCTGGGAATTCAGAAC
CCATCAACAGACAAGGCACCTAAAGGTCCCACAGGTGAAAGACCCCTG
GCTGCAGGGAAAGACCCTGGCCCCCCAGACCCAAAGAAAGCTCCGGAT
CCACCCACCCTGAAGAAAGATGCCAAAGCCCCTGCCTCAGAGAAAGGG
GATGGTACCCTGGCCCAACCCTCAACTAGCAGCCAAGGCCCCAAAGGA
GAGGGTGACAGGGGCGGGGGGCCCGCGGAGGGCAGTGCTGGGCCCCCG
GCAGCCCTGCCCCAGCAGACTGCGACACCTGAGACCAGCGTCAAGAAG
CCCAAGGCTGAGCAGGGAGCCTCAGGCAGCCAGGATCCTGGAAAGCCC
AGGGTGGGCAAGAAGGCAGCAGAGGGCCAAGCAGCAGCCAGGAGGGGC
TCACCTGCCTTTCTGCATAGCCCCAGCTGTCCTGCCATCATCTCCAGT
TCTGAGAAGCTGCTGGCCAAGAAGCCCCCAAGCGAGGCATCAGAGCTC
ACCTTTGAAGGGGTGCCCATGACCCACAGCCCCACGGATCCCAGGCCA
GCCAAGGCAGAAGAAGGAAAGAACATCCTGGCAGAGAGCCAGAAGGAA
GTGGGAGAGAAAACCCCAGGCCAGGCTGGCCAGGCTAAGATGCAAGGG
GACACCTCGAGGGGGATCGAGTTCCAGGCTGTTCCCTCAGAGAAATCC
GAGGTGGGGCAGGCCCTCTGTCTCACAGCCAGGGAGGAGGACTGCTTC
CAGATTTTGGATGATTGCCCGCCACCTCCGGCCCCCTTCCCTCACCGC
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TCCAAGGAGGCGCTCGGAGGTGGCAAGTTTGGGGCAGTCTGTACCTGC
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CAGACTCCCAAAGACAAGGAAATGGTGTTGCTGGAGATTGAGGTCATG
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AAGATGAGGGTTTTGCACCTGGACCTCAAGCCAGAGAACATCCTGTGT
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CGGAGGTATAACCCCAACGAGAAGCTGAAGGTGAACTTTGGGACCCCA
GAGTTCCTGTCACCTGAGGTGGTGAATTATGACCAAATCTCCGATAAG
ACAGACATGTGGAGTATGGGGGTGATCACCTACATGCTGCTGAGCGGC
CTCTCCCCCTTCCTGGGAGATGATGACACAGAGACCCTAAACAACGTT
CTATCTGGCAACTGGTACTTTGATGAAGAGACCTTTGAGGCCGTATCA
GACGAGGCCAAAGACTTTGTCTCCAACCTCATCGTCAAGGACCAGAGG
GCCCGGATGAACGCTGCCCAGTGTCTCGCCCATCCCTGGCTCAACAAC
CTGGCGGAGAAAGCCAAACGCTGTAACCGACGCCTTAAGTCCCAGATC
TTGCTTAAGAAATACCTCATGAAGAGGCGCTGGAAGAAAAACTTCATT
GCTGTCAGCGCTGCCAACCGCTTCAAGAAGATCAGCAGCTCGGGGGCA
CTGATGGCTCTGGGGGTCtgagcggccgc