Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active MYLK4 [1 – 388]

Enzyme description:- MYLK4 [1 - 388]

Clone number:- DU 37137

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 71, 385.32 daltons
Average Mass 71, 431.22 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.90

Purity:- 85 %

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, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

Assay buffer:-
50 mM Tris-HCl pH 7.5, 0.1 mM EGTA, 0.1 % 2–mercaptoethanol, 10 mM MgAc

Substrate:-
KKRPQRATSNVFA Final concentration: 300 μM

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Clone Data Sheet

MYLK4 [1 - 388]

Protein	MYLK4 [1 - 388]
Clone number	DU 37137
Species	Human
Accession number	NM_001012418.3
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MLKVKRLEEFNTCY** **NSNQLEKMAFFQCREEVEKVKCFLEKNSGDQDSRSRHNEAKEVWSNADL** **TERMPVKSKRTSALAVDIPAPPAPFDHRIVTAKQGAVNSFYTVSKTEIL** **GGGRFGQVHKCEETATGLKLAAKIIKTRGMKDKEEVKNEISVMNQLDHA** **NLIQLYDAFESKNDIVLVMEYVDGGELFDRIIDESYNLTELDTILFMKQ** **ICEGIRHMHQMYILHLDLKPENILCVNRDAKQIKIIDFGLARRYKPREK** **LKVNFGTPEFLAPEVVNYDFVSFPTDMWSVGVIAYMLLSGLSPFLGDND** **AETLNNILACRWDLEDEEFQDISEEAKEFISKLLIKEKSWRISASEALK** **HPWLSDHKLHSRLNAQKKKNRGSDAQDFVTK**
Native sequence	Amino acids M1 – K388 (end) of human MYLK4. Residue M232 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 - 229
Cloning sites	*Bam*H1 and *Not*1 sites of pGEX 6P-1

Nucleotide Sequence of insert

ggatccATGTTAAAAGTGAAGAGGCTGGAAGAATTCAACACGTGTTATA
ACAGCAACCAGCTGGAGAAAATGGCCTTTTTTCAGTGCAGGGAAGAGGT
GGAGAAAGTGAAGTGTTTTCTGGAAAAAAATTCTGGGGACCAGGATTCA
AGATCTAGACATAATGAGGCGAAGGAGGTGTGGTCAAACGCCGACCTGA
CGGAAAGGATGCCCGTCAAAAGCAAAAGGACATCAGCCCTCGCAGTTGA
CATCCCGGCTCCTCCGGCCCCATTTGATCATCGTATTGTGACAGCCAAG
CAAGGAGCGGTCAACAGCTTCTATACTGTGAGCAAGACAGAAATCCTAG
GAGGAGGGCGTTTCGGCCAGGTTCACAAGTGTGAGGAGACGGCCACAGG
TCTGAAGCTGGCAGCCAAAATCATCAAGACCAGAGGCATGAAGGACAAG
GAGGAGGTGAAGAACGAGATCAGCGTCATGAACCAGCTGGACCACGCGA
ACCTCATCCAGCTGTACGATGCCTTCGAGTCTAAGAACGACATTGTCCT
GGTCATGGAGTATGTGGATGGTGGGGAGCTGTTTGACCGCATCATCGAT
GAGAGCTACAATTTGACGGAGCTTGATACCATCCTGTTCATGAAGCAGA
TATGTGAGGGGATAAGGCACATGCATCAGATGTACATTCTCCACTTGGA
CCTGAAGCCTGAGAATATCCTGTGTGTGAATCGGGATGCTAAGCAAATA
AAAATTATTGATTTTGGATTGGCCAGAAGATACAAACCCAGAGAGAAGC
TGAAGGTGAACTTTGGAACCCCAGAATTTCTCGCCCCTGAAGTTGTGAA
CTATGATTTTGTTTCATTTCCCACTGACATGTGGAGTGTGGGGGTCATC
GCCTATATGCTACTTAGCGGTTTGTCGCCTTTCCTGGGTGACAATGATG
CTGAGACGCTGAACAACATCCTGGCCTGCAGGTGGGACTTAGAGGATGA
AGAATTTCAGGACATCTCGGAGGAGGCCAAGGAGTTCATCTCTAAGCTT
CTGATTAAGGAGAAGAGTTGGCGAATAAGTGCAAGCGAAGCTCTCAAGC
ACCCCTGGTTGTCAGACCACAAGCTCCACTCCAGACTCAATGCCCAGAA
GAAGAAGAATCGTGGCTCTGATGCCCAGGACTTTGTGACCAAAtaggcg
gccgc