Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active CLK3 [1 - 490]

Enzyme description:- CLK3 [1 - 490]

Clone number:- DU 58727

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N–terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 85, 358.01 daltons
Average Mass 85, 412.56 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 9.12

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, 10 mM DTT, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70 °C

Assay Buffer:-
50 mM Tris-HCl pH 7.5, 0.1mM EGTA, 10 mM DTT, 10 mM MgAc

Substrate:-
RNRYRDVSPFDHSR Final concentration: 300 uM

Clone Data Sheet

CLK3 [1 - 490]

Protein	CLK3 [1 - 490]
Clone number	DU 58727
Species	Human
Accession number	NM_003992.4
Tags	N-terminal GST
Baculovirus expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNK KFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKE RAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKM FEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKL VCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSD LEVLFQGPLGS**MHHCKRYRSPEPDPYLSYRWKRRRSYSREHEGR** **LRYPSRREPPPRRSRSRSHDRLPYQRRYRERRDSDTYRCEERSP** **SFGEDYYGPSRSRHRRRSRERGPYRTRKHAHHCHKRRTRSCSSA** **SSRSQQSSKRSSRSVEDDKEGHLVCRIGDWLQERYEIVGNLGEG** **TFGKVVECLDHARGKSQVALKIIRNVGKYREAARLEINVLKKIK** **EKDKENKFLCVLMSDWFNFHGHMCIAFELLGKNTFEFLKENNFQ** **PYPLPHVRHMAYQLCHALRFLHENQLTHTDLKPENILFVNSEFE** **TLYNEHKSCEEKSVKNTSIRVADFGSATFDHEHHTTIVATRHYR** **PPEVILELGWAQPCDVWSIGCILFEYYRGFTLFQTHENREHLVM** **MEKILGPIPSHMIHRTRKQKYFYKGGLVWDENSSDGRYVKENCK** **PLKSYMLQDSLEHVQLFDLMRRMLEFDPAQRITLAEALLHPFFA** **GLTPEERSFHTSRNPSR**
Native sequence	Amino acids M1 – R490 (end residue) of human CLK3. 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 - 228
Cloning sites	*Bam*H1 and *Not*1sites of pFastBac Dual.

Nucleotide sequence of insert

ggatccATGCATCACTGTAAGCGATACCGCTCCCCTGAACCAGAC
CCGTACCTGAGCTACCGATGGAAGAGGAGGAGGTCCTACAGTCGG
GAACATGAAGGGAGACTGCGATACCCGTCCCGAAGGGAGCCTCCC
CCACGAAGATCTCGGTCCAGAAGCCATGACCGCCTGCCCTACCAG
AGGAGGTACCGGGAGCGCCGTGACAGCGATACATACCGGTGTGAA
GAGCGGAGCCCATCCTTTGGAGAGGACTACTATGGACCTTCACGT
TCTCGTCATCGTCGGCGATCGCGGGAGAGGGGGCCATACCGGACC
CGCAAGCATGCCCACCACTGCCACAAACGCCGCACCAGGTCTTGT
AGCAGCGCCTCCTCGAGAAGCCAACAGAGCAGTAAGCGCAGCAGC
CGGAGTGTGGAAGATGACAAGGAGGGTCACCTGGTGTGCCGGATC
GGCGATTGGCTCCAAGAGCGATATGAGATTGTGGGGAACCTGGGT
GAAGGCACCTTTGGCAAGGTGGTGGAGTGCTTGGACCATGCCAGA
GGGAAGTCTCAGGTTGCCCTGAAGATCATCCGCAACGTGGGCAAG
TACCGGGAGGCTGCCCGGCTAGAAATCAACGTGCTCAAAAAAATC
AAGGAGAAGGACAAAGAAAACAAGTTCCTGTGTGTCTTGATGTCT
GACTGGTTCAACTTCCACGGTCACATGTGCATCGCCTTTGAGCTC
CTGGGCAAGAACACCTTTGAGTTCCTGAAGGAGAATAACTTCCAG
CCTTACCCCCTACCACATGTCCGGCACATGGCCTACCAGCTCTGC
CACGCCCTTAGATTTCTGCATGAGAATCAGCTGACCCATACAGAC
TTGAAACCAGAGAACATCCTGTTTGTGAATTCTGAGTTTGAAACC
CTCTACAATGAGCACAAGAGCTGTGAGGAGAAGTCAGTGAAGAAC
ACCAGCATCCGAGTGGCTGACTTTGGCAGTGCCACATTTGACCAT
GAGCACCACACCACCATTGTGGCCACCCGTCACTATCGCCCGCCT
GAGGTGATCCTTGAGCTGGGCTGGGCACAGCCCTGTGACGTCTGG
AGCATTGGCTGCATTCTCTTTGAGTACTACCGGGGCTTCACACTC
TTCCAGACCCACGAAAACCGAGAGCACCTGGTGATGATGGAGAAG
ATCCTAGGGCCCATCCCATCACACATGATCCACCGTACCAGGAAG
CAGAAATATTTCTACAAAGGGGGCCTAGTTTGGGATGAGAACAGC
TCTGACGGCCGGTATGTGAAGGAGAACTGCAAACCTCTGAAGAGT
TACATGCTCCAAGACTCCCTGGAGCACGTGCAGCTGTTTGACCTG
ATGAGGAGGATGTTAGAATTTGACCCTGCCCAGCGCATCACACTG
GCCGAGGCCCTGCTGCACCCCTTCTTTGCTGGCCTGACCCCTGAG
GAGCGGTCCTTCCACACCAGCCGCAACCCAAGCAGAtgagcggcc
gc