Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active CLK4 [128 - 481]

Enzyme description:- CLK4 [128 - 481]

Clone number:- DU 58744

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N–terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 68, 534.60 daltons
Average Mass 68, 578.88 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.44

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

CLK4 [128 - 481]

Protein	CLK4 [128 - 481]
Clone number	DU 58744
Species	Human
Accession number	NM_020666.2
Tags	N-terminal GST
Baculovirus expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNK KFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKE RAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKM FEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKL VCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSD LEVLFQGPLGS**SKSHRRKRSRSIEDDEEGHLICQSGDVLRARYE** **IVDTLGEGAFGKVVECIDHGMDGMHVAVKIVKNVGRYREAARSE** **IQVLEHLNSTDPNSVFRCVQMLEWFDHHGHVCIVFELLGLSTYD** **FIKENSFLPFQIDHIRQMAYQICQSINFLHHNKLTHTDLKPENI** **LFVKSDYVVKYNSKMKRDERTLKNTDIKVVDFGSATYDDEHHST** **LVSTRHYRAPEVILALGWSQPCDVWSIGCILIEYYLGFTVFQTH** **DSKEHLAMMERILGPIPQHMIQKTRKRKYFHHNQLDWDEHSSAG** **RYVRRRCKPLKEFMLCHDEEHEKLFDLVRRMLEYDPTQRITLDE** **ALQHPFFDLLKKK**
Native sequence	Amino acids S128 – K481 (end residue) of human CLK4. Residue S232 of the fusion protein is equivalent to S128 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

ggatccTCGAAGAGCCACCGAAGGAAAAGATCCAGGAGTATAGAG
GATGATGAGGAGGGTCACCTGATCTGTCAAAGTGGAGACGTTCTA
AGAGCAAGATATGAAATCGTGGACACTTTGGGTGAAGGAGCCTTT
GGCAAAGTTGTAGAGTGCATTGATCATGGCATGGATGGCATGCAT
GTAGCAGTGAAAATCGTAAAAAATGTAGGCCGTTACCGTGAAGCA
GCTCGTTCAGAAATCCAAGTATTAGAGCACTTAAATAGTACTGAT
CCCAATAGTGTCTTCCGATGTGTCCAGATGCTAGAATGGTTTGAT
CATCATGGTCATGTTTGTATTGTGTTTGAACTACTGGGACTTAGT
ACTTACGATTTCATTAAAGAAAACAGCTTTCTGCCATTTCAAATT
GACCACATCAGGCAGATGGCGTATCAGATCTGCCAGTCAATAAAT
TTTTTACATCATAATAAATTAACCCATACAGATCTGAAGCCTGAA
AATATTTTGTTTGTGAAGTCTGACTATGTAGTCAAATATAATTCT
AAAATGAAACGTGATGAACGCACACTGAAAAACACAGATATCAAA
GTTGTTGACTTTGGAAGTGCAACGTATGATGATGAACATCACAGT
ACTTTGGTGTCTACCCGGCACTACAGAGCTCCCGAGGTCATTTTG
GCTTTAGGTTGGTCTCAGCCTTGTGATGTTTGGAGCATAGGTTGC
ATTCTTATTGAATATTACCTTGGTTTCACAGTCTTTCAGACTCAT
GATAGTAAAGAGCACCTGGCAATGATGGAACGAATATTAGGACCC
ATACCACAACACATGATTCAGAAAACAAGAAAACGCAAGTATTTT
CACCATAACCAGCTAGATTGGGATGAACACAGTTCTGCTGGTAGA
TATGTTAGGAGACGCTGCAAACCGTTGAAGGAATTTATGCTTTGT
CATGATGAAGAACATGAGAAACTGTTTGACCTGGTTCGAAGAATG
TTAGAATATGATCCAACTCAAAGAATTACCTTGGATGAAGCATTG
CAGCATCCTTTCTTTGACTTATTAAAAAAGAAAtgagcggccgc