Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active CLK2 [138 – 499]

Enzyme description:- CLK2 [138 - 499]

Clone number:- DU 16987

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 1 mg/L

Calculated molecular mass:-
Monoisotopic 69, 507.17 daltons
Average Mass 69, 551.72 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.33

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, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -80 °C

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

Substrate:-
RNRYRDVSPFDHSR Final concentration: 300 ˜M

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Clone Data Sheet

CLK2 [138 - 499]

Protein	CLK2 [138 - 499]
Clone number	DU 16987
Species	Human
Accession number	NM_003993.2
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKW RNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNML GGCPKERAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFL SKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLY MDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQG WQATFGGGDHPPKSDLEVLFQGPLGS**RRAKSVEDDAEGHLI YHVGDWLQERYEIVSTLGEGTFGRVVQCVDHRRGGARVALK IIKNVEKYKEAARLEINVLEKINEKDPDNKNLCVQMFDWFD YHGHMCISFELLGLSTFDFLKDNNYLPYPIHQVRHMAFQLC QAVKFLHDNKLTHTDLKPENILFVNSDYELTYNLEKKRDER SVKSTAVRVVDFGSATFDHEHHSTIVSTRHYRAPEVILEL GWSQPCDVWSIGCIIFEYYVGFTLFQTHDNREHLAMMERIL GPIPSRMIRKTRKQKYFYRGRLDWDENTSAGRYVRENCKPL RRYLTSEAEEHHQLFDLIESMLEYEPAKRLTLGEALQHPFF ARLRAEPPNKLWDSSRDISR**
Native sequence	Amino acids R138 – R499 (end) of human CLK2. Residue R232 of the fusion protein is equivalent to R138 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*1 sites of pFastBAC GST

Nucleotide Sequence of insert

ggatccCGGAGAGCCAAGAGTGTAGAGGACGACGCTGAGGGCCACCTC
ATCTACCACGTCGGGGACTGGCTACAAGAGCGATATGAAATCGTTAGC
ACCTTAGGAGAGGGGACCTTCGGCCGAGTTGTACAATGTGTTGACCAT
CGCAGGGGTGGGGCTCGAGTTGCCCTGAAGATCATTAAGAATGTGGAG
AAGTACAAGGAAGCAGCTCGACTTGAGATCAACGTGCTAGAGAAAATC
AATGAGAAAGACCCTGACAACAAGAACCTCTGTGTCCAGATGTTTGAC
TGGTTTGACTACCATGGCCACATGTGTATCTCCTTTGAGCTTCTGGGC
CTTAGCACCTTCGATTTCCTCAAAGACAACAACTACCTGCCCTACCCC
ATCCACCAAGTGCGCCACATGGCCTTCCAGCTGTGCCAGGCTGTCAAG
TTCCTCCATGATAACAAGCTGACACATACAGACCTCAAGCCTGAAAAT
ATTCTGTTTGTGAATTCAGACTATGAGCTCACCTACAACCTAGAGAAG
AAGCGAGATGAGCGCAGTGTGAAGAGCACAGCTGTGCGGGTGGTAGAC
TTTGGCAGTGCCACCTTTGACCATGAGCACCATAGCACCATTGTCTCC
ACTCGCCATTACCGAGCACCAGAAGTCATCCTTGAGTTGGGCTGGTCA
CAGCCTTGTGATGTGTGGAGTATAGGCTGCATCATCTTTGAATACTAT
GTGGGATTCACCCTCTTCCAGACCCATGACAACAGAGAGCATCTAGCC
ATGATGGAAAGGATCTTGGGTCCTATCCCTTCCCGGATGATCCGAAAG
ACAAGAAAGCAGAAATATTTTTACCGGGGTCGCCTGGATTGGGATGAG
AACACATCAGCTGGGCGCTATGTTCGTGAGAACTGCAAACCGCTGCGG
CGGTATCTGACCTCAGAGGCAGAGGAACACCACCAGCTCTTCGATCTG
ATTGAAAGCATGCTAGAGTATGAACCAGCTAAGCGGCTGACCTTGGGT
GAAGCCCTTCAGCATCCTTTCTTCGCCCGCCTTCGGGCTGAGCCGCCC
AACAAGTTGTGGGACTCCAGTCGGGATATCAGTCGGtgagcggccgc