MRC PPU Reagents and Services

Standard Operating Procedure

Preparation of CLK2 [138 – 499]

Enzyme description:- CLK2 [138 - 499]

Clone number:- DU 31001

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal GST

Purification method:- GSH Sepharose

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 %

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 uM

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Clone Data Sheet

CLK2 [138 - 499]

Protein CLK2 [138 - 499]

Clone number DU 16987

Species Human

Accession number NM_001294338.2

Tags N-terminal GST

Baculovirus 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