Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active CLK1 [129 - 484]

Enzyme description:- CLK1 [129 - 484]

Clone number:- DU 27352

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 68, 555.83 daltons
Average Mass 68, 600.03 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.57

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:- -70 °C

Assay:- Standard filter binding assay

Assay buffer:-
50 mM Tris-HCl pH 7.5, 0.1 mM EGTA, 10 mM DTT, 2 mM manganese chloride

Substrate:-
RNRYRDVSPFDHSR Final concentration: 300 uM

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Clone Data Sheet

CLK1 [129 - 484]

Protein	CLK1 [129 - 484]
Clone number	DU 27352
Species	Human
Accession number	NM_004071.3
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSM**HGKSHRRKRTRSV** **EDDEEGHLICQSGDVLSARYEIVDTLGEGAFGKVVECIDHKAGGRHVAV** **KIVKNVDRYCEAARSEIQVLEHLNTTDPNSTFRCVQMLEWFEHHGHICI** **VFELLGLSTYDFIKENGFLPFRLDHIRKMAYQICKSVNFLHSNKLTHTD** **LKPENILFVQSDYTEAYNPKIKRDERTLINPDIKVVDFGSATYDDEHHS** **TLVSTRHYRAPEVILALGWSQPCDVWSIGCILIEYYLGFTVFPTHDSKE** **HLAMMERILGPLPKHMIQKTRKRKYFHHDRLDWDEHSSAGRYVSRRCKP** **LKEFMLSQDVEHERLFDLIQKMLEYDPAKRITLREALKHPFFDLLKKSI**

Native sequence	Amino acids H129 – I484 (end) of human CLK1. Residue H233 of the fusion protein is equivalent to H129 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 pGEX6P-1

Nucleotide Sequence Of Insert

ggatccatgCATGGGAAGAGTCACCGAAGGAAAAGAACCAGGAGTGTAGAGGATGATGA
GGAGGGTCACCTGATCTGTCAGAGTGGAGACGTACTAAGTGCAAGATATGAAATTGTTG
ATACTTTAGGTGAAGGAGCTTTTGGAAAAGTTGTGGAGTGCATCGATCATAAAGCGGGA
GGTAGACATGTAGCAGTAAAAATAGTTAAAAATGTGGATAGATACTGTGAAGCTGCTCG
CTCAGAAATACAAGTTCTGGAACATCTGAATACAACAGACCCCAACAGTACTTTCCGCT
GTGTCCAGATGTTGGAATGGTTTGAGCATCATGGTCACATTTGCATTGTTTTTGAACTA
TTGGGACTTAGTACTTACGACTTCATTAAAGAAAATGGTTTTCTACCATTTCGACTGGA
TCATATCAGAAAGATGGCATATCAGATATGCAAGTCTGTGAATTTTTTGCACAGTAATA
AGTTGACTCACACAGACTTAAAGCCTGAAAACATCTTATTTGTGCAGTCTGACTACACA
GAGGCGTATAATCCCAAAATAAAACGTGATGAACGCACCTTAATAAATCCAGATATTAA
AGTTGTAGACTTTGGTAGTGCAACATATGATGACGAACATCACAGTACATTGGTATCTA
CAAGACATTATAGAGCACCTGAAGTTATTTTAGCCCTAGGGTGGTCCCAACCATGTGAT
GTCTGGAGCATAGGATGCATTCTTATTGAATACTATCTTGGGTTTACCGTATTTCCAAC
ACACGATAGTAAGGAGCATTTAGCAATGATGGAAAGGATTCTTGGACCTCTACCAAAAC
ATATGATACAGAAAACCAGGAAACGTAAATATTTTCACCACGATCGATTAGACTGGGAT
GAACACAGTTCTGCCGGCAGATATGTTTCAAGACGCTGTAAACCTCTGAAGGAATTTAT
GCTTTCTCAAGATGTTGAACATGAGCGTCTCTTTGACCTCATTCAGAAAATGTTGGAGT
ATGATCCAGCCAAAAGAATTACTCTCAGAGAAGCCTTAAAGCATCCTTTCTTTGACCTT
CTGAAGAAAAGTATAtaggcggccgc