Certificate of Analysis

ProQinase TARGETING CANCER

Recombinant Proteins

CLK1

CDC-like kinase 1

Recombinant Human Active Protein Kinase

HGNC Symbol: CLK1

Synonyms: CLK, CLK4, STY

Product No.: 0447-0000-1

Lot: 001

Description: Human CLK1, full length, amino acids M_1-I_{484} (as in NCBI/Protein entry NP_004062.2), N-terminal GST-HIS_6 fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: CLK1 Lot 001, was confirmed as CLK1 by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 86,695 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

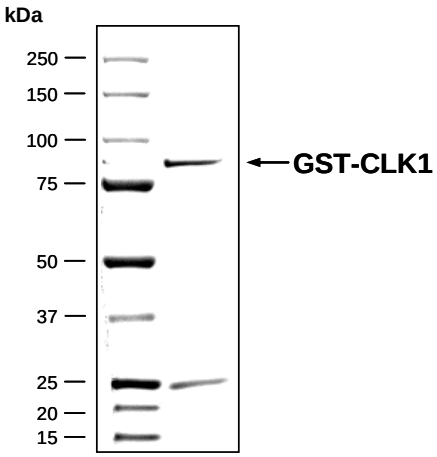
Storage buffer: 50 mM TRIS-HCl pH 8.0, 100 mM NaCl, 5 mM DTT, 4 mM reduced glutathione, 20 % glycerol

Storage temperature: -80°C
For complete recovery, mix well and spin before use. Product must not be stored in diluted solutions, aliquots below 10µl are not advisable. Avoid repeated freeze-thaw cycles!

Protein concentration: 0.139 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

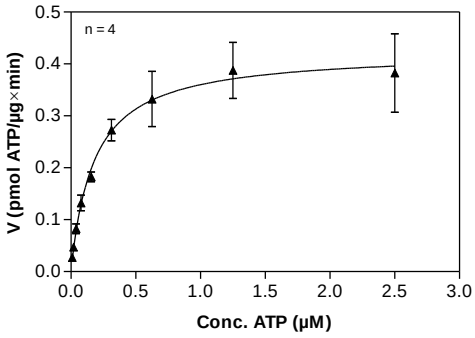
Biochemical Parameters:
Specific kinase activity (P_i transfer): 0.4 pmol/µg × min
ATP-K_M: 0.2 µM

CLK1 Lot 001:
Coomassie stain



2.0 µg GST-CLK1

CLK1 Lot 001:
Determination of V_{max} and K_M value for ATP



Determination of K_M value & Specific activity:
- Assay conditions:
  60 mM HEPES-NaOH, pH 7.5
  3 mM $MgCl_2$
  3 mM $MnCl_2$
  3 µM Na-orthovanadate
  1.2 mM DTT
  50 µg/ml $PEG_{20.000}$
  ATP (variable)
  Substrate: RS-peptide, 40 µg/ml
  Kinase: 1 µg/ml
- Filter binding assay

MSPH membrane (Millipore)

Additional assay technology:
CLK1 Lot 001 was also successfully tested by ProQinase for the use with the ADP-Glo™ Kinase assay from Promega
ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details

ProQinase
TARGETING CANCER

CLK1

Product No.: 0447-0000-1

GST-CLK1 Recombinant Fusion Protein Amino Acid Sequence

1	MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID	60
61	GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV	120
121	DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK	180
181	KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD PMGHHHHHHG RRRASVAAGI	240
241	LVPRGSPGLD GICSRMRHSK RTYCPDWDDK DWDYGKWRSS SSHKRRKRSH SSAHENKRCK	300
301	YNHSKMCDSH YLESRSINEK DYHSRRYIDE YRNDYTQGCE PGHRQRDHES RYQNHSSKSS	360
361	GRSGRSSYKS KHRIHHSTSH RRSHGKSHRR KRTRSVEDDE EGHLICQSGD VLSARYEIVD	420
421	TLGEGAFGKV VECIDHKAGG RHVAVKIVKN VDRYCEAARS EIQVLEHLNT TDPNSTFRCV	480
481	QMLEWFEHHG HICIVFELLG LSTYDFIKEN GFLPFRLDHI RKMAYQICKS VNFLHSNKLT	540
541	HTDLKPENIL FVQSDYTEAY NPKIKRDERT LINPDIKVVD FGSATYDDEH HSTLVSTRHY	600
601	RAPEVILALG WSQPCDVWSI GCILIEYYLG FTVFPTHDSK EHLAMMERIL GPLPKHMIQK	660
661	TRKRKYFHHD RLDWDEHSSA GRYVSRRCKP LKEFMLSQDV EHERLFDLIQ KMLEYDPAKR	720
721	ITLREALKHP FFDLLKKSI	780

1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **blue**: CLK1 **boxed**: variation from RefSeq

CLK1 wt[1] Amino Acid Sequence

1	MRHSKRTYCP DWDDKDWDYG KWRSSSSHKR RKRSHSSAQE NKRCKYNHSK MCDSHYLESR	60
61	SINEKDYHSR RYIDEYRNDY TQGCEPGHRQ RDHESRYQNH SSKSSGRSGR SSYKSKHRIH	120
121	HSTSHRRSHG KSHRRKRTRS VEDDEEGHLI CQSGDVLSAR YEIVDTLGEG AFGKVVECID	180
181	HKAGGRHVAV KIVKNVDRYC EAARSEIQVL EHLNTTDPNS TFRCVQMLEW FEHHGHICIV	240
241	FELLGLSTYD FIKENGFLPF RLDHIRKMAY QICKSVNFLH SNKLTHTDLK PENILFVQSD	300
301	YTEAYNPKIK RDERTLINPD IKVVDFGSAT YDDEHHSTLV STRHYRAPEV ILALGWSQPC	360
361	DVWSIGCILI EYYLGFTVFP THDSKEHLAM MERILGPLPK HMIQKTRKRK YFHHDRLDWD	420
421	EHSSAGRYVS RRCKPLKEFM LSQDVEHERL FDLIQKMLEY DPAKRITLRE ALKHPFFDLL	480
481	KKSI	540

blue: CLK1 sequence expressed in recombinant protein **Red**: variant in recombinant protein

[1]NCBI/Protein accession number NP_004062.2

Recombinant Proteins