

Certificate of Analysis

CLK1, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-920, 14-920-K, 14-920M

Parent Lot # D12EP004N

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: N-terminal 6His-tagged, recombinant, human CLK1, amino acids 130-end expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography.

Purity 86% by SDS-PAGE and Coomassie blue staining. MW = 45kDa.

Specific Activity (Parent lot# D12EP004N): 848U/mg, where one unit of CLK1, active activity is defined as 1nmol phosphate incorporated into 200µM (ERM₂PRKRQGSVRRRV) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.445mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

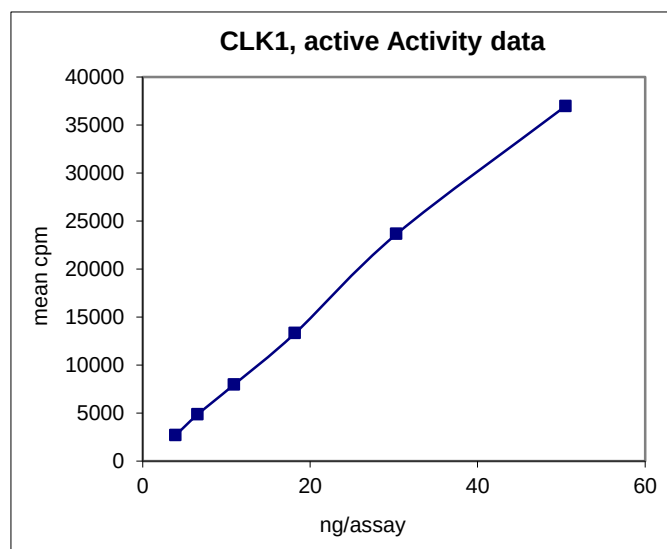
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

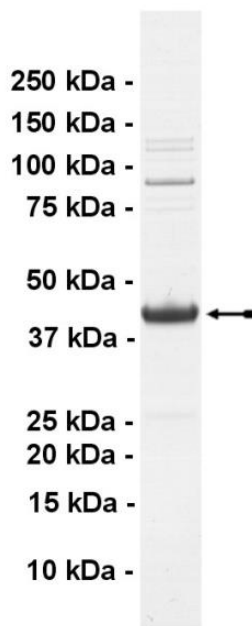
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 2–50ng of this lot of enzyme phosphorylated 200µM (ERM₂PRKRQGSVRRRV) in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS Tryptic Fingerprint: Confirmed identity as CLK1 with the translated sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of CLK1, active.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **Na₃VO₄:** Use at a final assay concentration of 1mM. Prepare a 100mM stock and add 0.25μl of stock per assay point.
3. **Na-β-glycerophosphate:** Use at a final assay concentration of 5mM. Prepare a 1M stock and add 0.125μl of stock per assay point.
4. **(ERM_RPRKRQGSVRRRV):** Use at a final assay concentration of 200μM. Prepare a 2mM stock and add 2.5μl of stock per assay point.
5. **CLK1:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 2–50ng per assay point.
6. **[γ-³³P]ATP:** 2.5 x MgAc/[γ-³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required).

Assay Procedure (96 well plate format):

1. Add 5μl of 5 x reaction buffer per assay to wells.
2. Add 2.5μl of **(ERM_RPRKRQGSVRRRV)**.
3. Add **2.5μl (2–50ng) CLK1**.
4. Add 4.625μl of dH₂O.
5. Add 0.25μl Na₃VO₄
6. Add 0.125μl Na-β-glycerophosphate
7. Add 10μl of diluted [γ-³³P]ATP mixture.
8. Incubate for 10 minutes at 30°C.
9. Stop the reaction by adding 5μl of 3% phosphoric acid.
10. Transfer a 10μl aliquot onto the appropriate area of a **P30 Filtermat**.
11. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
12. Wash the filtermat once for 2 minutes with methanol.
13. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
14. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1μl of 30% phosphoric acid.

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CLK1 Sequence Information

<u>Protein</u>	human CLK1
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	G31 of the recombinant protein is equivalent to G130 of human CLK1
<u>Accession number</u>	GenBank NM_004071

Recombinant CLK1 amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF GKSHRRKRTR SVEDDEEGHL ICQSGDVLSA
61 RYEIVDTLGE GAFGKVEECI DHKAGGRHVA VKIVKNVDYR CEAAARSEIQV LEHLNTTDPN
121 STFRVCVQMLE WFEHHGHICI VFELLGLSTY DFIKENGFLP FRLDHIRKMA YQICKSVNFL
181 HSNKLTHTDL KPENILFVQS DYTEAYNPKEI KRDERTLINP DIKVVDGSA TYDDEHHSTL
241 VSTRHYRAPE VILALGWSQP CDVWSIGCIL IEYYLGFTVF PTHDSKEHLA MMERILGPLP
301 KHMIQKTRKR KYFHHDRLDW DEHSSAGRYV SRRCKPLKEF MLSQDVEHER LFDLIQKMLE
361 YDPAKRITLR EALKHPFFDL LKKSII

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Recombinant CLK1 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tatttttcagg gcgccatgga tccggaattc gggaagagtc accgaaggaa aagaaccagg
121 agtgttagagg atgatgagga gggtcacctg atctgtcaga gtggagacgt actaagtgcg
181 agatatgaaa ttgttgatac tttagggtgaa ggagcttttg gaaaagtgtg ggagtgcacg
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1141 ctgaagaaaa gtatatag

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