

Certificate of Analysis

CLK4, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-917, 14-917-K, 14-917M

Parent Lot # D12DP004N

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 CLK4 amino acids 128-end expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography.

Purity 87.3% by SDS-PAGE and Coomassie blue staining. MW = 46kDa.

Specific Activity (Parent lot# D12DP004N): 289U/mg, where one unit of CLK4, active activity is defined as 1nmol phosphate incorporated into 200 μ M (YRRAVPPSPSLSRHSSPHQS(p)EDEEE) per minute at 30°C with a final ATP concentration of 100 μ M.

Formulation: 0.704mg/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.

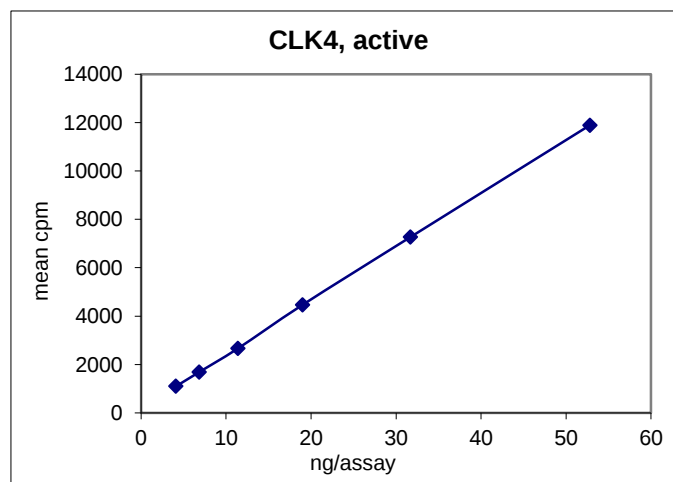
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

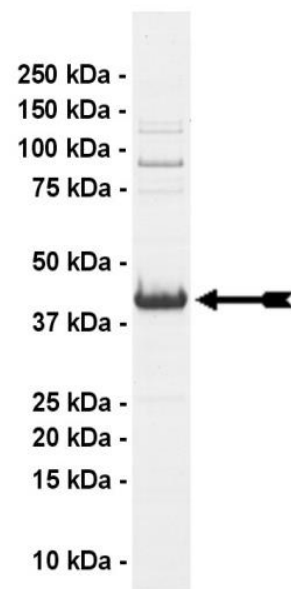
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 4–53ng of this lot of enzyme phosphorylated 200 μ M (YRRAVPPSPSLSRHSSPHQS(p)EDEEE) 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 CLK4 with the translated sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3 μ g of CLK4, active.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **(YRRAAVPPSPSLSRHSSPHQS(p)EDEEE):** Use at a final assay concentration of 200 μ M. Prepare a 2mM stock and add 2.5 μ l of stock per assay point.
3. **CLK4, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 4–53ng per assay point.
4. **[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 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 μ l of (YRRAAVPPSPSLSRHSSPHQS(p)EDEEE).
3. Add **2.5 μ l (4–53ng) CLK4, active.**
4. Add 5 μ l of dH₂O.
5. Add 10 μ l of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
8. Transfer a 10 μ l aliquot onto the appropriate area of a **P30 Filtermat.**
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1 μ l of 30% phosphoric acid.

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CLK4 Sequence Information

<u>Protein</u>	Human CLK4
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	S31 of the recombinant protein is equivalent to S128 of human CLK4
<u>Accession number</u>	GenBank NM_020666

Recombinant CLK4 amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF SKSHRRKRSR SIEDDEEGHL ICQSGDVLRA
61 RYEIVDTLGE GAFGKVVECI DHGMDGMHVA VKIVKNVGRY REAARSEIQV LEHLNSTDPN
121 SVFRCVQMLE WFDHHGHVCI VFELLGLSTY DFIKENSFLP FQIDHIRQMA YQICQSINFL
181 HHNKLTHSDL KPNILFVKS DYVVKYNSKM KRDERLTKNT DIKVVDGSA TYDDEHHSTL
241 VSTRHYRAPE VILALGWSQP CDVWSIGCIL IEYYLGFTVF QTHDSKEHLA MMERILGPIP
301 QHMIQKTRKR KYFHHNQLDW DEHSSAGRYV RRRCKPLKEF MLCHDEEHEK LFDLVRRLME
361 YDPTQRITLD EALQHPFFDL LKKK

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Recombinant CLK4 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tatttttcagg gcgcatgga tccggaattc tcgaagagcc accgaaggaa aagatccagg
121 agtatagagg atgatgagga gggtcacctg atctgtcaaa gtggagacgt tctaagagca
181 agatatgaaa tcgtggacac tttgggtgaa ggagcctttg gcaaagtgt agagtgcatt
241 gatcatggca tggatggcat gcatgtagca gtgaaaatcg taaaaaatgt aggccgttac
301 cgtgaagcag ctctgttcaga aatccaagta ttagagcact taaatagtac tgatcccaat
361 agtgtcttcc gatgtgtcca gatgctagaa tggtttgatc atcatgggtca tgtttgtatt
421 gtgtttgaac tactgggact tagtacttac gatttcatta aagaaaacag ctttctgccca
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1081 tatgatccaa ctcaaagaat taccttggat gaagcattgc agcatcctt ctttgactta
1141 ttaaaaaaga aatga

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