

Certificate of Analysis

CLK3, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-724, 14-724-K, 14-724M

Parent Lot # D8EN014U

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, full length human CLK3, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 83.5% by SDS-PAGE and Coomassie blue staining. MW = 63kDa.

Specific Activity (Parent lot# D8EN014U): 1378U/mg, where one unit of CLK3, active activity is defined as 1nmol phosphate incorporated into 250μM (ERM₂PRKRQGSVRRRV) per minute at 30°C with a final ATP concentration of 100μM.

Formulation: 1.028mg/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.

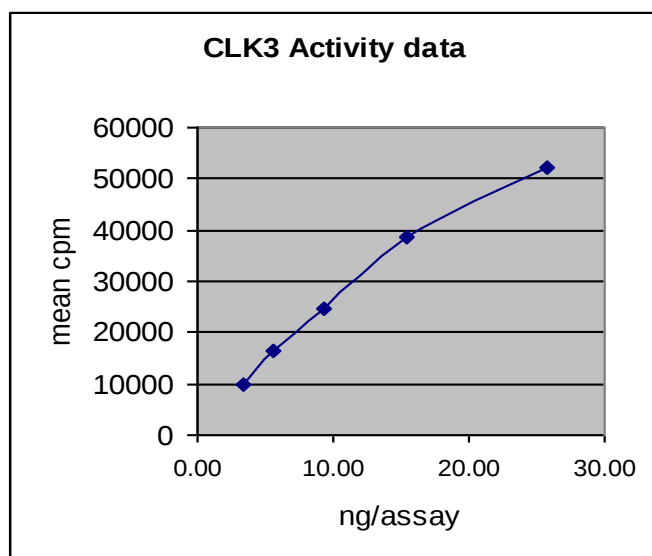
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

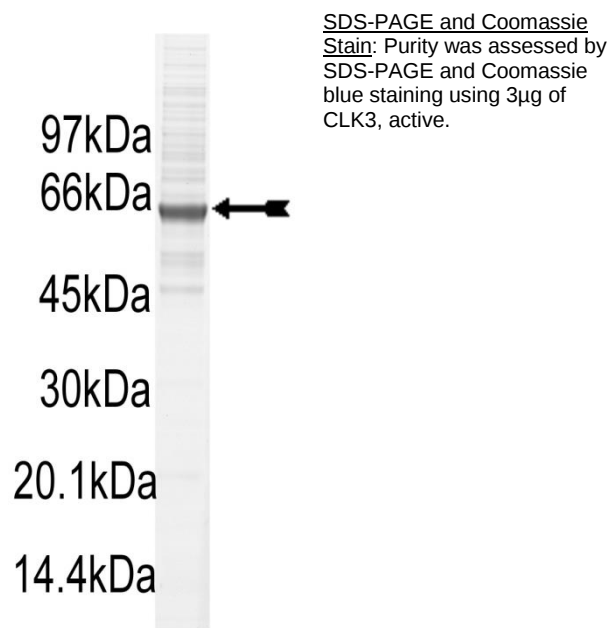
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 3–25ng of this lot of enzyme phosphorylated 250μM (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 CLK3 with the translated native sequence listed on page three.



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS-NaOH pH7.0, 1mM EDTA.
2. **(ERMRPRKRQGSVRRRV):** Use at a final assay concentration of 2500µM. Make up a 2.5mM stock. Add 2.5µl of stock per assay point.
3. **NaCl:** Use at a final assay concentration of 30mM. Make a 3M stock. Add 0.25µl of stock per assay point.
4. **CLK3, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 3–25ng per assay point.
5. **[γ-³³P]ATP:** 2.5 x magnesium acetate/[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 **(ERMRPRKRQGSVRRRV)**.
3. Add **2.5µl (3–25ng) CLK3, active**.
4. Add 0.25µl of 3M NaCl.
5. Add 4.75µl of dH₂O.
6. Add 10µl of diluted [γ-³³P]ATP mixture.
7. Incubate for 10 minutes at 30°C.
8. Stop the reaction by adding 5µl of 3% phosphoric acid.
9. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat**.
10. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
11. Wash the filtermat once for 2 minutes with methanol.
12. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
13. 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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CLK3 Sequence Information

Protein	Human CLK3
Tags	N-terminal 6His
Native sequence	M37 of recombinant sequence is equivalent to M1 of native human CLK3
Accession number	GenBank NM_003992. The CLK3 construct contains the translational conflicts T132S and G133S with respect to the accession number given. These are both reported in GB BC019881.

Recombinant CLK3 amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF KGLRRQMHHHC KRYRSPEPDP YLSYRWKRRR
61 SYSREHEGRL RYPSRREPPP RRSRSRSHDR LPYQRRYRER RSDTYRCEE RSPSFGEDYY
121 GPSRSRHHRR SRERGPYRTR KHAHHCHKRR TRSCSSASSR SQQSSKRSSR SVEDDKGHL
181 VCRIGDWLQE RYEIVGNLGE GTFGKVVECL DHARGKSQVA LKIIRNVGKY REAARLEINV
241 LKKIKEKDKE NKFLCVLMSD WFNFGHMC I AFELGKNTF EFLKENNFQ YPLPHVRHMA
301 YQLCHALRFL HENQLTHTDL KPENILFVNS EFETLYNEHK SCEEKSVKNT SIRVADFGSA
361 TFDHEHHHTTI VATRHYRPPE VILELGWAQP CDVWSIGCIL FEYYRGFTLF QTHENREHLV
421 MMEKILGPIP SHMIHRTRKQ KYFYKGGLVW DENSSDGRYV KENCKPLKSY MLQDSLEHVQ
481 LFDLMRRMLE FDPAQRLT LA EALLHPFFAG LTPEERSFHT SRNPSR

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Recombinant CLK3 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgccatgga tccggaattc aaaggcctac gtcgacaaat gcatcactgt
121 aagcgatacc gctccctga accagaccg tacctgagct accgatggaa gaggaggagg
181 tcctacagtc gggaaacatga agggagactg cgatacccg cccgaaggga gcctcccca
241 cgaagatctc ggtccagaag ccatgaccgc ctgccctacc agaggaggta cggggagcgc
301 cgtgacagcg atacataccg gtgtgaagag cggagtccat cctttggaga ggactactat
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721 ctcaaaaaaa tcaaggagaa ggacaaagaa aacaagttcc tgtgtgtctt gatgtctgac
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1201 tttgagtact accggggctt cacactcttc cagaccacag aaaaccgaga gcacctggtg
1261 atgatggaga agatcctagg gcccatccca tcacacatga tccaccgtac caggaagcag
1321 aaatatttct acaaaggggg cctagtttgg gatgagaaca gctctgacgg ccggtatgtg
1381 aaggagaact gcaaacctct gaagagttac atgctccaag actccctgga gcacgtgcag
1441 ctgtttgacc tgatgaggag gatgttagaa tttgaccctg cccagcgcat cacactggcc
1501 gaggccctgc tgcaccctt ctttgctggc ctgaccctg aggagcggtc cttccacacc
1561 agccgcaacc caagcagatg a

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