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Certificate of Analysis

CLK2, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-774, 14-774-K 14-774M
Parent Lot # 1635817

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 GST-tagged, recombinant, human, CLK2, amino acids 138–end, expressed by baculovirus in *Sf*21 cells. Purified using glutathione agarose. Purity 81.3% by SDS-PAGE and Coomassie blue staining. MW = 70.4kDa.

Specific Activity (Parent lot# 1635817): 1203U/mg, where one unit of CLK2, active activity is defined as 1nmol phosphate incorporated into 20µM Phospho GS2 (YRRAAVPPSPSLSRHSSPHQS(p)EDEEE) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.962mg/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.

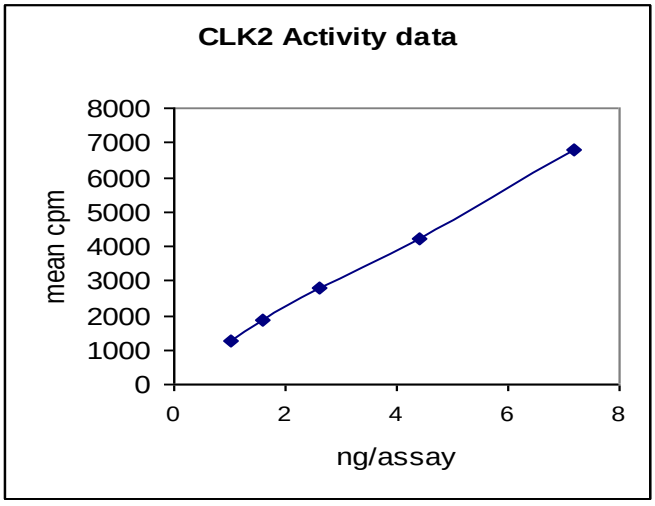
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

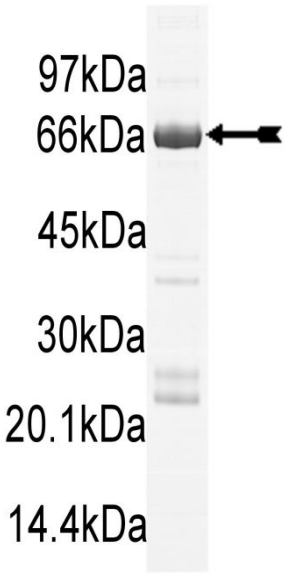
Quality Control Testing

Kinase Assay: 1.0–7.2ng of this lot of enzyme phosphorylated 20µM Phospho GS2 (YRRAAVPPSPSLSRHSSPHQS(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 product identity as CLK2 with the translated native sequence listed on page three.

SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of CLK2, active



Eurofins Pharma Discovery Services UK Limited
Gemini Crescent
Dundee Technology Park
DUNDEE
DD2 1SW United Kingdom
T | +44 (0)1382 561600
F | +44 (0)1382 561601
www.eurofins.com/pharmadiscovery

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS-NaOH pH7.0, 1mM EDTA.
2. **PhosphoGS2 (YRRAAVPPSPSLSRHSSPHQS(p)EDEEE):** Use at a final assay concentration of 20µM. Make up a 200µM stock. Add 2.5µl of stock per assay point.
3. **CLK2, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 1.0–7.2ng per assay point.
4. **[γ-^{33}P]ATP:** 2.5X magnesium acetate/[γ^{33}P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ^{33}P]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 **(YRRAAVPPSPSLSRHSSPHQS(p)EDEEE)**.
3. Add **2.5µl (1.0–7.2ng) CLK2, active**.
4. Add 5µl of dH_2O.
5. Add 10µl of diluted [γ-^{33}P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5µl of 3% phosphoric acid.
8. Transfer a 10µl 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 1µl of 30% phosphoric acid.

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CLK2 Sequence Information

Protein Human CLK2

Tags *N*-terminal GST

Native sequence R237 of recombinant sequence is equivalent to R138 of native human CLK2

Accession number GenBank NM_003993

Recombinant CLK2 amino acid sequence:

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  1 MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
 61 GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LEVLFQGPEF KGLRRQRRAK
241 SVEDDAEGHL IYHVGDWLQE RYEIVSTLGE GTFGRVVQCV DHRRGGARVA LKIIKNVEKY
301 KEAARLEINV LEKINEKDPD NKNLCVQMFD WFDYHGHMCI SFELLGLSTF DFLKDNNYLP
361 YPIHQVRHMA FQLCQAVKFL HDNKLTHTDL KPENILFVNS DYELTYNLEK KRDERSVKST
421 AVRVVDFGSA TFDHEHHSTI VSTRHYRAPE VILELGWSQP CDVWSIGCII FEYYVGFTLF
481 QTHDNREHLA MMERILGPIP SRMIRKTRKQ KYFYRGRLDW DENTSAGRYV RENCKPLRRY
541 LTSEAEEHHQ LFDLIESMLE YEPAKRLTLG EALQHPFFAR LRAEPPNKLW DSSRDISR
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Recombinant CLK2 nucleotide sequence:

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   1 atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaacccac tcgacttctt
  61 ttggaatatc ttgaagaaaa atatgaagag catttgtatg agcgcgatga aggtgataaa
 121 tggcgaaaca aaaagtttga attgggtttg gagtttccca atcttcctta ttatattgat
 181 ggtgatgtta aattaacaca gtctatggcc atcatacgtt atatagctga caagcacaac
 241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcggttttg
 301 gatattagat acggtgtttc gagaattgca tatagtaaag actttgaaac tctcaaagtt
 361 gattttctta gcaagctacc tgaaatgctg aaaatgttcg aagatcgttt atgtcataaa
 421 acatatttaa atggtgatca tgtaacccat cctgacttca tgttgtatga cgctcttgat
 481 gttgttttat acatggaccc aatgtgcctg gatgcgttcc caaaattagt ttgttttaaa
 541 aaacgtattg aagctatccc acaaattgat aagtacttga aatccagcaa gtatatagca
 601 tggcctttgc agggctggca agccacgttt ggtggtggcg accatcctcc aaaatcggat
 661 ctggaagttc tgttccaggg gcccgaattc aaaggcctac gtcgacaacg gagagccaag
 721 agtgtagagg acgacgctga gggccacctc atctaccacg tcggggactg gctacaagag
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1021 tcctttgagc ttctgggcct tagcaccttc gatttcctca aagacaacaa ctacctgccc
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1141 catgataaca agctgacaca tacagacctc aagcctgaaa atattctgtt tgtgaattca
1201 gactatgagc tcacctacaa cctagagaag aagcgagatg agcgcagtgt gaagagcaca
1261 gctgtgcggg tggtagactt tggcagtgcc acctttgacc atgagcacca tagcaccatt
1321 gtctccactc gccattaccg agcaccagaa gtcatccttg agttgggctg gtcacagcct
1381 tgtgatgtgt ggagtatagg ctgcatcatc tttgaatact atgtgggatt caccctcttc
1441 cagacccatg acaacagaga gcatctagcc atgatggaaa ggatcttggg tcctatccct
1501 tcccggatga tccgaaagac aagaaagcag aaatattttt accggggtcg cctggattgg
1561 gatgagaaca catcagctgg gcgctatgtt cgtgagaact gcaaaccgct gcggcggtat
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1621 ctgacctcag aggcagagga acaccaccag ctcttcgatc tgattgaaag catgctagag
1681 tatgaaccag ctaagcggct gaccttgggt gaagcccttc agcatccttt cttcgcccgc
1741 cttcgggctg agccgcccaa caagttgtgg gactccagtc gggatatcag tcggtga
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Reviewed and approved by site quality representative.