

Certificate of Analysis

PhKy2, active

(Recombinant enzyme expressed in *E. coli* cells)

Item # 14-698, 14-698-K, 14-698M

Parent Lot # D8CN057U

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, PhKy2, amino acids (1–301) containing the mutation F290L, expressed by *E.coli* cells. Purified using glutathione-agarose. Purity 89.1% by SDS-PAGE and Coomassie blue staining. MW = 61.37kDa.

Specific Activity (Parent lot# D8CN057U): 134U/mg, where one unit of PhKy2, active activity is defined as 1nmol phosphate incorporated into 250µM (KKLNRTLSFAEPG) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 1.138mg/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.

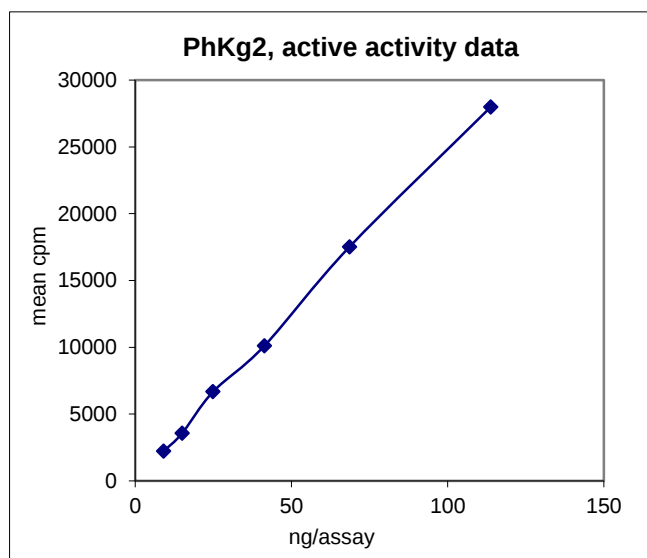
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

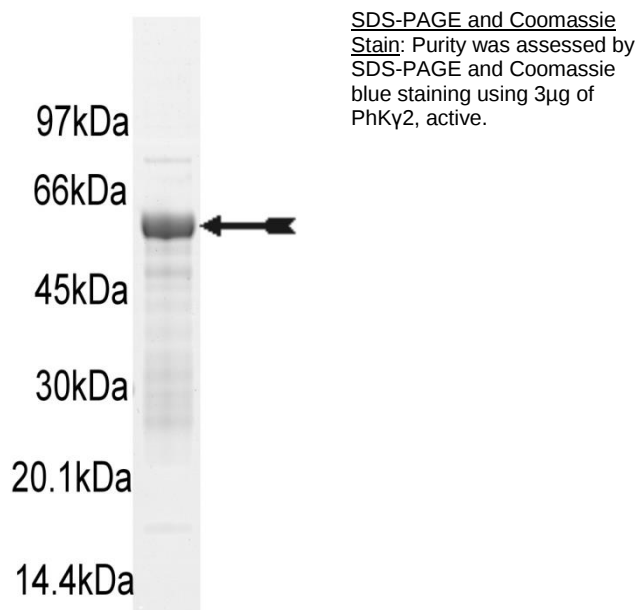
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 9–114ng of this lot of enzyme phosphorylated 250µM (KKLNRTLSFAEPG) 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 PhKy2 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. **(KKLNRTLSFAEPG):** Use at a final concentration of 250 μ M. Make up a 2.5mM stock. Use 2.5 μ l of stock per assay point.
3. **PhK γ 2, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 9–114ng per assay point.
4. **[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 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 μ l of **(KKLNRTLSFAEPG)**.
3. Add **2.5 μ l (9–114ng), PhK γ 2 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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PhKy2 Sequence Information

<u>Protein</u>	Human PhKy2
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	M230 of recombinant sequence is equivalent to M1 of human PhKy2
<u>Accession number</u>	GenBank NM_000294. The recombinant protein contains the amino acid substitution F290L with respect to this accession number. Inhibition studies on recombinant PhKy2 F290L exhibit a trend similar to that observed with the wild-type PhK holoenzyme using the inhibitors K252a, Bis-5 and Ro 318220 (Davies <i>et al.</i> , (2000), Biochem. J. 351 , 95-105; Elliott, <i>et al.</i> , (1990), Biochem. Biophys. Res. Commun. 171 ,148-154)

Recombinant PhKy2 amino acid sequence:

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1  MSPILGYWKI KGLVQPTRL L LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
61  GDVKLTQ SMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSR IA YSKDFETLKV
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAI PQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LVPRGSPEFM TLDVGPEDEL
241 PDWAAAKEFY QKYDPKDVIG RGVSSVVRRC VHRATGHEFA VKIMEVTAER LSPEQLEEV R
301 EATRRETHIL RQVAGHPHII TLIDSYESSS FMFLVFDLMR KGELFDYL TE KVALSEKETR
361 SIMRSLLEAV SFLHANNIVH RDLKPENILL DDNMQIRLSD FGFSCHLEPG EKLRELCGTP
421 GYLAPEILKC SMDETHPGYG KEVDLWACGV ILFTLLAGSP PFWHRRQILM LRMIMEGQYQ
481 FSSPEWDDRS STVKDLISRL LQVDPEARLT AEQALQHPLF ERCEGSQPWN

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Recombinant PhKy2 nucleotide sequence:

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1  atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaaccac tcgacttctt
61  ttggaatata ttgaagaaaa atatgaagag catttgatat agcgcgatga aggtgataaa
121 tggcgaaaca aaaagtttga attgggtttg gagtttccca atcttcctta ttatatgat
181 ggtgatgtta aattaacaca gtctatggcc atcatagctg atatatagct caagcacaa
241 atgttggttg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcggttttg
301 gatattagat acggtgtttc gagaattgca tatagtaaag actttgaaac tctcaaagtt
361 gattttctta gcaagctacc tgaatgctg aaaatgttcg aagatcgttt atgtcataaa
421 acatatatta atggtgatca tgaaccat cctgacttca tgttgatatg cgtcttgat
481 gttgttttat acatggaccc aatgtgcctg gatgcgttcc caaaattagt ttgttttaaa
541 aaacgtattg aagctatccc acaaattgat aagtacttga aatccagcaa gtatatagca
601 tggcctttgc agggctggca agccacgttt ggtggtggcg accatcctcc aaaatcggat
661 ctggttccgc gtggatcccc ggaattcatg acgctggacg tggggccgga ggatgagctg
721 cccgactggg ccgccgcaa agagttttac cagaagtacg accctaagga cgtcatcggc
781 agaggagtga gctctgtggt ccgccgttgt gttcatcgag ctactggcca cgagtttgcg
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901 gaagccacac ggcgagagac acacatcctt cgccaggtcg ccggccaccc ccacatcatc
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1021 aaggagagagc tgtttgacta tctcacagag aagggtggccc tctctgaaaa ggaaaccagg
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1201 ttcgggttct cctgccactt ggaacctggc gagaagcttc gagagttgtg tgggaccca
1261 gggatatctag cgccagagat ccttaaatgc tccatggatg aaaccaccc aggctatggc
1321 aaggaggtcg acctctgggc ctgtgggggtg atcttgttca cactcctggc tggctcgcca
1381 cccttctggc accggcggca gatcctgatg ttacgcatga tcatggaggg ccagtaccag

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1441 ttcagttccc ccgagtggga tgaccgttcc agcactgtca aagacctgat ctccaggctg
1501 ctgcaggtgg atcctgaggc acgcctgaca gctgagcagg ccctacagca cccctctttt
1561 gagcgttggtg aaggcagcca accctggaac taa
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