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Certificate of Analysis

PhKγ2, 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, PhKγ2, 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 PhKγ2, 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.

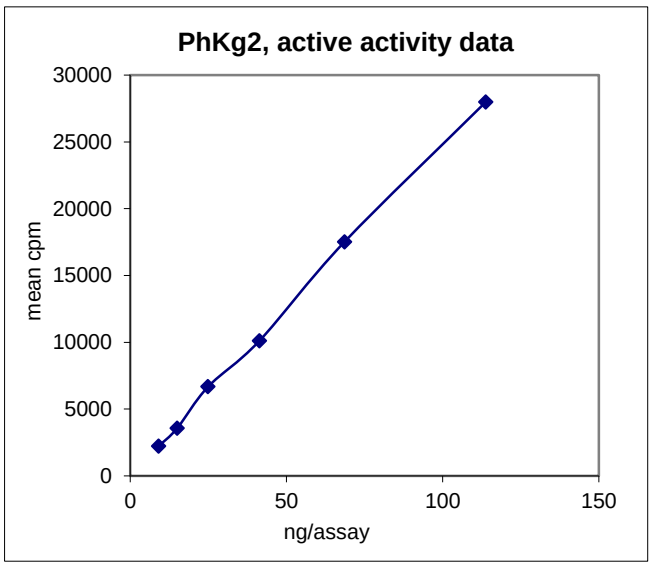
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

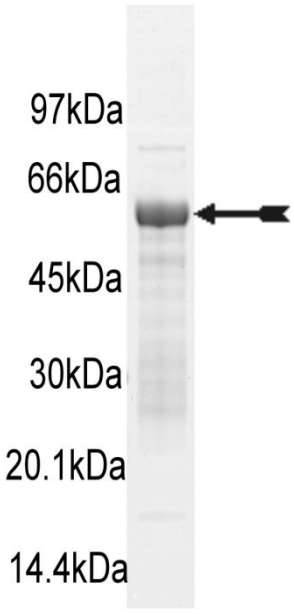
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

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 PhKγ2 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 PhKγ2, active.

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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 250µM. Make up a 2.5mM stock. Use 2.5µl of stock per assay point.
3. **PhKγ2, 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-^{33}P]ATP:** 2.5 x 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 **(KKLNRTLSFAEPG)**.
3. Add **2.5µl (9–114ng), PhKγ2 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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PhKγ2 Sequence Information

Protein Human PhKγ2

Tags *N*-terminal GST

Native sequence M230 of recombinant sequence is equivalent to M1 of human PhKγ2

Accession number GenBank NM_000294. The recombinant protein contains the amino acid substitution F290L with respect to this accession number. Inhibition studies on recombinant PhKγ2 F290L exhibit a trend similar to that observed with the wild-type PhK holoenzyme using the inhibitors K252a, Bis-5 and Ro 318220 (Davies *et al*., (2000), Biochem. J. **351**, 95-105; Elliott, *et al*., (1990), Biochem. Biophys. Res. Commun. **171**,148-154)

Recombinant* PhKγ2 *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 LVPRGSPEFM TLDVGPEDEL
 241 PDWAAAKEFY QKYDPKDVIG RGVSSVVRRC VHRATGHEFA VKIMEVTAER LSPEQLEEVR
 301 EATRRETHIL RQVAGHPHII TLIDSYESSS FMFLVFDLMR KGELFDYLTE 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* PhKγ2 *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
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 421 acatatttaa atggtgatca tgtaacccat cctgacttca tgttgtatga cgctcttgat
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 601 tggcctttgc agggctggca agccacgttt ggtggtggcg accatcctcc aaaatcggat
 661 ctggttccgc gtggatcccc ggaattcatg acgctggacg tggggccgga ggatgagctg
 721 cccgactggg ccgccgccaa agagttttac cagaagtacg accctaagga cgtcatcggc
 781 agaggagtga gctctgtggt ccgccgttgt gttcatcgag ctactggcca cgagtttgcg
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 901 gaagccacac ggcgagagac acacatcctt cgccaggtcg ccggccaccc ccacatcatc
 961 accctcatcg attcctacga gtcttctagc ttcatgttcc tggtgtttga cctgatgcgg
1021 aagggagagc tgtttgacta tctcacagag aaggtggccc tctctgaaaa ggaaaccagg
1081 tccatcatgc ggtctctgct ggaagcagtg agctttctcc atgccaacaa cattgtgcat
1141 cgagatctga agcccgagaa tattctccta gatgacaata tgcagatccg actttcagat
1201 ttcgggttct cctgccactt ggaacctggc gagaagcttc gagagttgtg tgggacccca
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1381 cccttctggc accggcggca gatcctgatg ttacgcatga tcatggaggg ccagtaccag
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1441 ttcagttccc ccgagtggga tgaccgttcc agcactgtca aagacctgat ctccaggctg
1501 ctgcaggtgg atcctgaggc acgcctgaca gctgagcagg ccctacagca ccccctcttt
1561 gagcgttgtg aaggcagcca accctggaac taa
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Reviewed and approved by site quality representative.