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Certificate of Analysis

PhKγ1, active
(Recombinant enzyme expressed in Sf21 insect cells)
Item # 16-018, 16-018-K, 16-018M
Parent Lot # WAF0020

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 PhKγ1 full length expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose.
Purity 37% by SDS-PAGE and Coomassie blue staining. MW = 72kDa.

Specific Activity (Parent lot# WAF0020): 63U/mg, where one unit of PhKγ1 activity is defined as 1nmol phosphate incorporated into 250µM KKLNRTLSFAEPG per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.44mg/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.

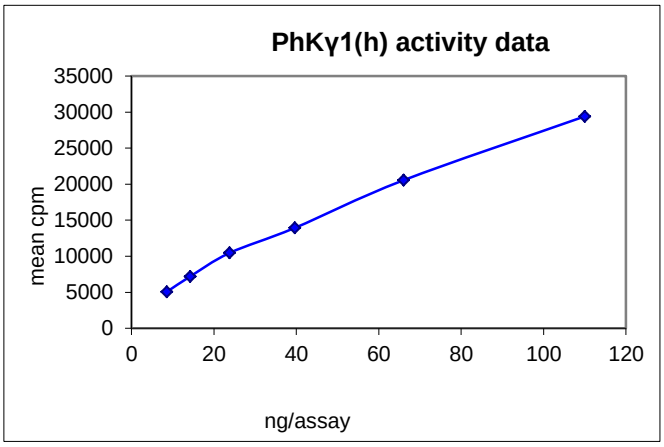
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

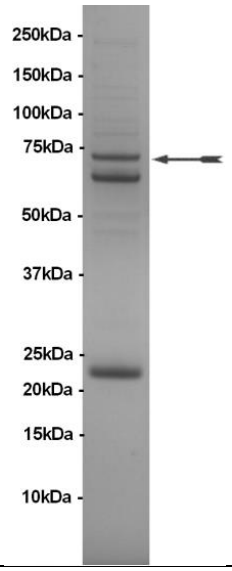
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 8.55–110ng 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 PhKγ1 with the translated sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of PhKγ1, 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 assay concentration of 250µM. Prepare a 2.5mM stock and add 2.5µl of stock per assay point.
3. **PhKγ1, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 8.55–110ng per assay point.
4. **[γ-^{33}P]ATP:** 2.5 x MgAc/[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 (8.55–110ng) PhKγ1, active.**
4. Add 5.0µl of dH_2O.
5. Add 10µl of diluted [γ-^{33}P]ATP mixture.
6. Incubate for 30 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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PhKγ1, active Sequence Information

Protein	Human PhKγ1
Tags	*N*-terminal GST
Native sequence	M230 of the recombinant protein is equivalent to M1 of human PhKγ1
Accession number	GenBank NM_006213.2

Recombinant PhKγ1 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 LVPRGSKEFM TRDEALPDSH
241 SAQDFYENYE PKEILGRGVS SVVRRCIHKP TSQEYAVKVI DVTGGGSFSP EEVRELREAT
301 LKEVDILRKV SGHPNIIQLK DTYETNTFFF LVFDLMKRGE LFDYLTEKVT LSEKETRKIM
361 RALLEVICTL HKLNIVHRDL KPENILLDDN MNIKLTDFGF SCQLEPGERL REVCGTPSYL
421 APEIIECSMN EDHPGYGKEV DMWSTGVIMY TLLAGSPPFW HRKQMLMLRM IMSGNYQFGS
481 PEWDDYSDTV KDLVSRFLVV QPQNRYTAEE ALAHPFFQQY LVEEVRHFSP RGKFKVIALT
541 VLASVRIYYQ YRRVKPVTRE IVIRDPYALR PLRRLIDAYA FRIYGHWVKK GQQQNRAALF
601 ENTPKAVLLS LAEEDY
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Recombinant PhKγ1 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
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1321 gacatgtgga gcactggcgt catcatgtac acgctgctgg ccggctcccc gcccttctgg
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1441 cccgagtggg atgattactc ggacaccgtg aaggacctgg tctcccgatt cctggtggtg
1501 caaccccaga accgctacac agcggaagag gccttggcac acccccttct ccagcagtac
1561 ttggtggagg aagtgcggca cttcagcccc cgggggaagt tcaaggtgat cgctctgacc
1621 gtgctggctt cagtgcggat ctactaccag taccgccggg tgaagcctgt gacccgggag
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1681 atcgtcatcc gagaccccta tgccctccgg cctctgcgcc ggctcatcga cgcctacgct
1741 ttccgaatct atggccactg ggtgaagaag gggcagcagc agaaccgggc agccctttc
1801 gagaacacac ccaaggccgt gctcctctcc ctggccgagg aggactactg a
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Reviewed and approved by site quality representative.