Certificate of Analysis
PHKG1, 10 µg

Recombinant Human Phosphorylase Kinase Gamma Subunit 1, GST-tagged

Part Number: PV3853
Lot Number: 830447F
Immediate Storage: -80°C
Shipping Conditions: dry ice

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Description:

Recombinant Human Full-Length protein, GST-tagged, expressed in insect cells. No special measures were taken to activate this kinase.

Specific Activity:

430 nmoles of phosphate transferred to MC peptide substrate (RQMSFRL) per minute per mg of total protein at 30°C. Activity determined at a final protein concentration of 3.33 µg/mL.

Concentration:

0.36 mg/mL total protein as measured using the Bradford protein assay with BSA as a standard.

Calculated **4,970 nM**.

Storage and Handling:

For maximum recovery please spin prior to use. Aliquots of the 5 ug, 10ug and 20ug sizes of kinase are not recommended as materials can be used in original packaging until exhausted. For larger sizes, the number of freeze/thaws may be reduced by preparing aliquots, aliquots below 20 µL are not recommended. **Please never store a kinase diluted.** If properly stored at −80°C, this product is guaranteed for 6 months from date of purchase.

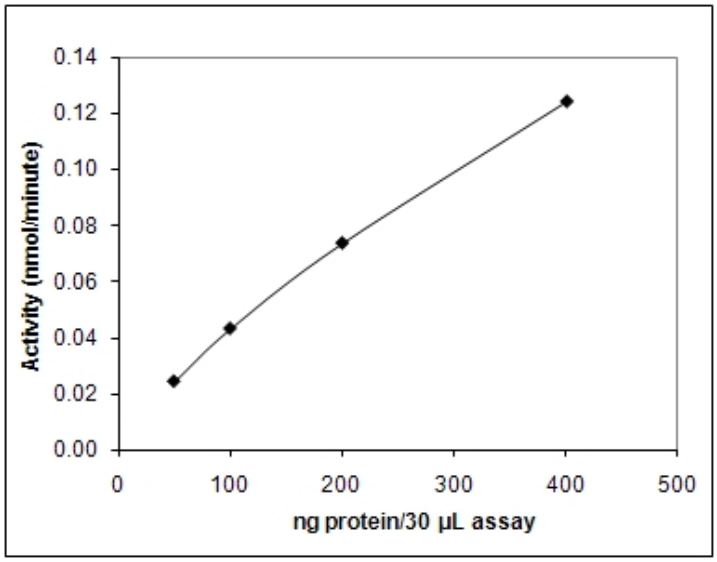
Protein not stable at room temperature, activity decreases by 30% after 8 hours. Stable for at least 48 hours at 4°C.

Storage Buffer:

50 mM Tris (pH 7.5), 150 mM NaCl, 0.5 mM EDTA, 0.02% Triton® X–100, 2 mM DTT and 50% Glycerol.

QUALITY ASSURANCE

PHKG1 Activity Graph



Dilution Buffer:

20 mM Tris (pH 7.5), 0.02% Triton® X−100, 0.1 mg/mL BSA, 2 mM DTT, 0.5 mM Na_3VO_4 and 10% Glycerol.

Assay Conditions:

PHKG1 was pre-diluted in enzyme dilution buffer and assayed in 25 mM Tris (pH 7.5), 10 mM $MgCl_2$, 0.5 mM EGTA, 0.5 mM Na_3VO_4, 5 mM β−glycerophosphate, 2.5 mM DTT, 0.01% Triton® X–100, 200 µM ATP, 200 µM MC peptide substrate (RQMSFRL) and trace [^{32}P]-γ-ATP for 10 minutes at 30°C.

Gel Information for PHKG1

Page Description: The SDS-PAGE and/or Native PAGE were run on 4-20% Tris-Glycine Novex® gels (Catalog #: EC6025BOX).

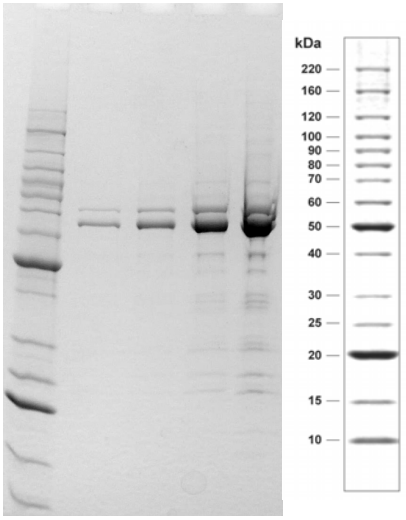
Lane 1: Invitrogen™ BenchMark™ Protein Ladder (Catalog #: 10747-012).

Lane 2: 1 µg PHKG1

Lane 3: 2 µg PHKG1

Lane 4: 5 µg PHKG1

Lane 5: 10 µg PHKG1



Purity:

60% as determined by a Coomassie® blue stained SDS-PAGE gel.

Molecular Weight:

72.5 kDa. Calculated from the protein sequence(s).

Mass Spectrometry:

PHKG1 was subjected to proteolytic digest followed by mass spec analysis. The resulting MS/MS data verified PHKG1 identity by comparison against the amino acid sequence(s) of the recombinant protein.

Protein sequence alignment with reference sequence(s)

GenBank Accession Number: NP_006204

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  1 MAPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL GST TAG
  1 MAPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL IVGN PHKG1
  1 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- NP_006204

101 DIRYGVSRIA YSKDFETLKV DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK KRIEAIPQID KYLKSSKYIA
101 DIRYGVSRIA YSKDFETLKV DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK KRIEAIPQID KYLKSSKYIA
  1 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

201 WPLQGWQATF GGGDHPPKSD LVPR
201 WPLQGWQATF GGGDHPPKSD LVPRHNQTSL YKKAGTMTRD EALPDSHSAQ DFYENYEPKE ILGRGVSSVV RRCIHKPTSQ EYAVKVIDVT GGGSFSPEEV
  1 ---------- ---------- ---------- ------MTRD EALPDSHSAQ DFYENYEPKE ILGRGVSSVV RRCIHKPTSQ EYAVKVIDVT GGGSFSPEEV

224
301 RELREATLKE VDILRKVSGH PNIIQLKDTY ETNTFFFLVF DLMKRGELFD YLTEKVTLSE KETRKIMRAL LEVICTLHKL NIVHRDLKPE NILLDDNMNI
 65 RELREATLKE VDILRKVSGH PNIIQLKDTY ETNTFFFLVF DLMKRGELFD YLTEKVTLSE KETRKIMRAL LEVICTLHKL NIVHRDLKPE NILLDDNMNI

224
401 KLTDFGFSCQ LEPGERLREV CGTPSYLAPE IIECSMNEDH PGYGKEVDMW STGVIMYTLL AGSPPFWHRK QMLMLRMIMS GNYQFGSPEW DDYSDTVKDL
165 KLTDFGFSCQ LEPGERLREV CGTPSYLAPE IIECSMNEDH PGYGKEVDMW STGVIMYTLL AGSPPFWHRK QMLMLRMIMS GNYQFGSPEW DDYSDTVKDL

224
501 VSRFLVVQPQ NRYTAEEALA HPFFQQYLVE EVRHFSPRGK FKV IALTVLA SVRIYYQYRR VKPVTREIVI RDPYALRPLR RLIDAYAFRI
265 VSRFLVVQPQ NRYTAEEALA HPFFQQYLVE EVRHFSPRGK FKV IALTVLA SVRIYYQYRR VKPVTREIVI RDPYALRPLR RLIDAYAFRI

224
601 QNRAALFENT PKAVLLSLAE EDY.
365 QNRAALFENT PKAVLLSLAE EDY
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* highlighted residues denote differences from the reference protein sequence(s).

Becky. Baker, QA Engineer III Date: 22/Apr/2013