

Certificate of Analysis

PKG1 α , active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-688, 14-688-K, 14-688M

Parent Lot # 2147495

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 6His-tagged, recombinant, full length PKG1 α , expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 86.7% by SDS-PAGE and Coomassie blue staining. MW = 80kDa.

Specific Activity (Parent lot# 2147495): 10980U/mg, where one unit of PKG1 α activity is defined as 1nmol phosphate incorporated into 200 μ M PAKtide (RRRLSFAEPG) per minute at 30°C with a final ATP concentration of 100 μ M.

Formulation: 1.163mg/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.

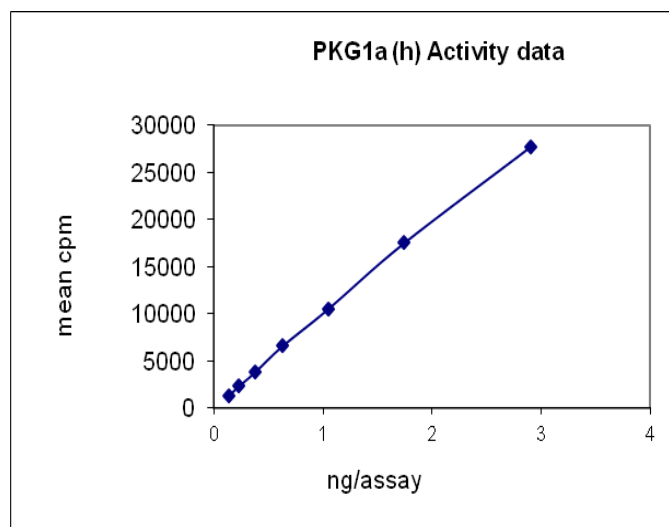
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

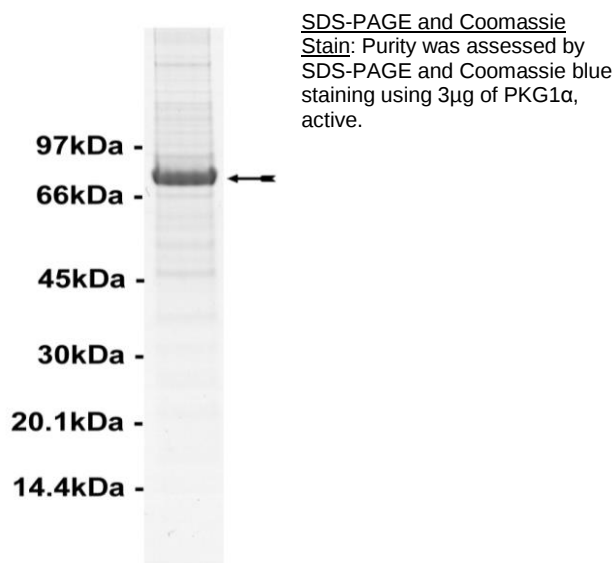
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 0.1–2.9ng of this lot of enzyme phosphorylated 200 μ M PAKtide (RRRLSFAEPG) 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 PKG1 α 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. **PAKtide (RRRLSFAEPG):** Use at a final assay concentration of 200 μ M. Make a 2mM stock. Add 2.5 μ l of stock per assay point.
3. **cGMP:** Use at a final assay concentration of 10 μ M. Make a 100 μ M stock. Add 2.5 μ l of stock per assay point.
4. **PKG1 α , active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.1–2.9ng per assay point.
5. **[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:

1. Add 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 μ l of **PAKtide (RRRLSFAEPG)**.
3. Add 2.5 μ l of 100 μ M **cGMP**.
4. Add **2.5 μ l (0.1–2.9ng) PKG1 α , active**.
5. Add 2.5 μ l of dH₂O.
6. Add 10 μ l of diluted [γ -³³P]ATP mixture.
7. Incubate for 10 minutes at 30°C.
8. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
9. Transfer a 10 μ l aliquot onto the appropriate area of a **P30 Filtermat**.
10. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
11. Wash the filtermat once for 2 minutes with methanol.
12. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
13. 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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PKG1 Sequence Information

Protein	Human PKG1
Tags	N-terminal 6His
Native sequence	M31 of the recombinant protein is equivalent to M1 of native human PKG1 α
Accession number	GenBank D45864

Recombinant PKG1 α amino acid sequence:

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1 MSYYHHHHH DYDIPTTENL YFQGAMDPEF MSELEEDFAK ILMLKEERIK ELEKRLSEKE
61 EEIQELKRKL HKCQSVLPVP STHIGPRTR AQGISAEPQT YRSFHDLRQA FRKFTKSERS
121 KDLIKEAILD NDFMKNLELS QIQEIVDCMY PVEYGKDSCI IKEGDVGS LV YVMEDGKVEV
181 TKEGVKLCTM GPGKVFGELA ILYNCTRTAT VKTLVNVKLW AIDRQCFQTI MMRTGLIKHT
241 EYMEFLKSVP TFQSLPEEIL SKLADVLEET HYENGEYIIR QGARGDTFFI ISKGTNVNTR
301 EDSPSEDPVF LRTLKGKDFW GEKALQGEDV RTANVIAAEA VTCLVIDRDS FKHLIGGLDD
361 VSNKAYEDAE AKAKYEAEEA FFANLKLSDF NIIDTLGVGG FGRVELVQLK SEESKTFAMK
421 ILKKRHIVDT RQQEHIRSEK QIMQGAHSDF IVRLYRTFKD SKYLYMLMEA CLGGELWTIL
481 RDRGSFEDST TRFYTACVVE AFAYLHSGKI IYRDLKPENL ILDHRYAKL VDFGFAKKIG
541 FGKKTWTF CG TPEYVAPEII LNKGHDISAD YWSLGILMYE LLTGSPPFSG PDPMKTYNII
601 LRGIDMIEFP KKIAKNAANL IKKLCRDNPS ERLGNLKNV KDIQKHKWE GFNWEGLRKG
661 TLPPIIPSV ASPTDTSNFD SFPEDNDEPP PDDNSGWDID F
  
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Recombinant PKG1 α nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg ggcctatgga tccggaattc atgagcgagc tagaggaaga ctttgccaag
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1621 tttggaaaga aaacatggac tttttgtggg actccagagt atgtagcccc agagatcatc
  
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1681 ctgaacaaag gccatgacat ttcagccgac tactgggtcac tgggaatcct aatgtatgaa
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1801 ttgaggggga ttgacatgat agaatttcca aagaagattg ccaaaaatgc tgctaattta
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2101 ttctaa
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