

## Certificate of Analysis

### PKG1 $\beta$ , active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-650, 14-650-K, 14-650M

Parent Lot # 1593237

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 $\beta$  expressed by baculovirus in Sf21 insect cells. Purified using Ni<sup>2+</sup>-NTA agarose. Purity 66.2% by SDS-PAGE and Coomassie blue staining. MW = 81.6kDa.

**Specific Activity (Parent lot# 1593237):** 12500U/mg, where one unit of PKG1 $\beta$ , active activity is defined as 1nmol phosphate incorporated into 200 $\mu$ M PAKtide (RRRLSFAEPG) per minute at 30°C with a final ATP concentration of 100 $\mu$ M.

**Formulation:** 0.971mg/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.

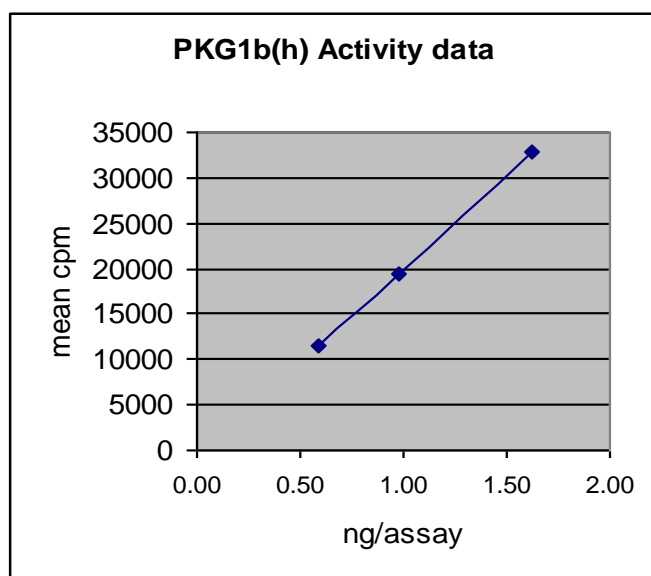
**Storage and Stability:** On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**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.

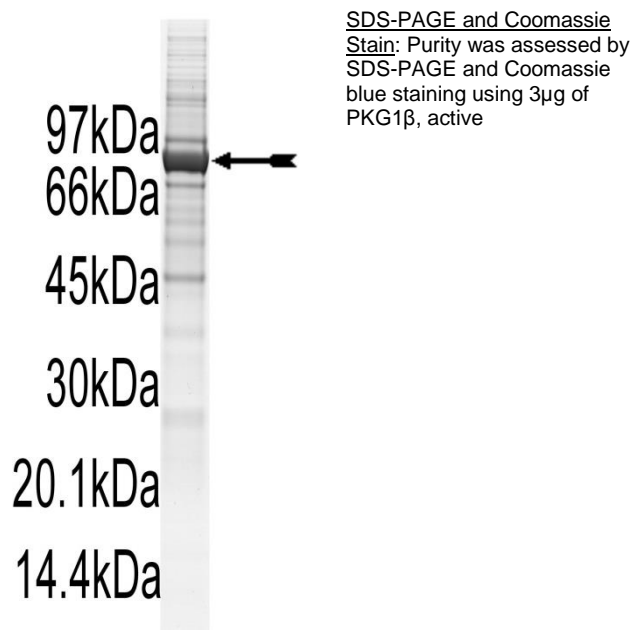
**FOR IN VITRO RESEARCH USE ONLY  
NOT FOR USE IN HUMANS OR ANIMALS**

### Quality Control Testing

**Kinase Assay:** 0.6–1.6ng of this lot of enzyme phosphorylated 200 $\mu$ 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 product identity as PKG1 $\beta$  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 $\mu$ M. Make a 2mM stock. Add 2.5 $\mu$ l of stock per assay point.
3. **cGMP:** Use at a final assay concentration of 10 $\mu$ M. Make a 100 $\mu$ M stock. Add 2.5 $\mu$ l of stock per assay point.
4. **PKG1 $\beta$  active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.6–1.6ng per assay point.
5. **[ $\gamma$ -<sup>33</sup>P]ATP:** 2.5 x magnesium acetate/[ $\gamma$ -<sup>33</sup>P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [ $\gamma$ -<sup>33</sup>P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

#### Assay Procedure:

1. Add 5 $\mu$ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 $\mu$ l of **PAKtide (RRRLSFAEPG)**.
3. Add 2.5 $\mu$ l of 100 $\mu$ M cGMP.
4. Add **2.5 $\mu$ l (0.6–1.6ng) PKG1 $\beta$ , active**.
5. Add 2.5 $\mu$ l of dH<sub>2</sub>O.
6. Add 10 $\mu$ l of diluted [ $\gamma$ -<sup>33</sup>P]ATP mixture.
7. Incubate for 10 minutes at 30°C.
8. Stop the reaction by adding 5 $\mu$ l of 3% phosphoric acid.
9. Transfer a 10 $\mu$ 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 $\mu$ l of 30% phosphoric acid.

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### PKG1 $\beta$ Sequence Information

<b><u>Protein</u></b>	Human PKG1 $\beta$
<b><u>Tags</u></b>	N-terminal 6His
<b><u>Native sequence</u></b>	M31 of the recombinant protein is equivalent to M1 of native human PKG1 $\beta$
<b><u>Accession number</u></b>	GenBank NM_006258

#### ***Recombinant PKG1 $\beta$ amino acid sequence:***

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1 MSYYHHHHH DYDIPTTENL YFQGAMDPEF MGTLRDLQYA LQEKIEELRQ RDALIDELEL
61 ELDQKDELIQ KLQNELDKYR SVIRPATQQA QKQSASTLQG EPRTKRQAIS AEPTAFDIQD
121 LSHVTLFPYP KSPQSKDLIK EAILDNDFMK NLELSQIQEI VDCMYPVEYG KDSCIIKEGD
181 VGSLVYVMED GKVEVTKGV KLCTMGPQV FGELAILYNC TRTATVKTLV NVKLWAIDRQ
241 CFQTIMMRTG LIKHTEYMEF LKSVPTFQSL PEEILSKLAD VLEETHYENG EYIIRQGARG
301 DTFFIISKGT VNVTREDSPTS EDPVFLRTLK KGDWFGEKAL QGEDVRTANV IAAEAVTCLV
361 IDRDSFKHLI GGLDDVSNKA YEDAEAKAKY EAEAAFFANL KLSDFNIIDT LGVGGFGRVE
421 LVQLKSEESK TFAMKILKKR HIVDTRQQEH IRSEKQIMQG AHSDFIVRLY RTFKDSKYLY
481 MLMEACLGG E LWTILRDRGS FEDSTTRFYT ACVVEAFAYL HSKGIIYRDL KPENLILDHR
541 GYAKLVDFGF AKKIGFGKKT WFCGTPEYV APEIILNKGH DISADYWSLG ILMYELLTGS
601 PPFSGPDPMK TYNIIIRGID MIEFPKIKAK NAANLIKKLC RDNPSERLGN LKNGVKDIQK
661 HKWFEFGNWE GLRKGTLTPP IIPSVASPTD TSNFDSFPED NDEPPPDDNS GWDIDF
    
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#### ***Recombinant PKG1 $\beta$ nucleotide sequence:***

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgccatgga tccggaattc atgggcacct tgcgggattt acagtacgcg
121 ctccaggaga agatcgagga gctgaggcag cgggatgctc tcatcgacga gctggagctg
181 gagttggatc agaaggacga actgatccag aagctgcaga acgagctgga caagtaccgc
241 tcggtgatcc gaccagccac ccagcaggcg cagaagcaga gcgcgagcac cttgcagggc
301 gagccgcgca ccaagcggca ggcgatctcc gccgagccca cgccttcga catccaggat
361 ctacgcatg tgaccctgcc cttctacccc aagagcccac agtccaagga tcttataaag
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541 gtggggtcac tgggtatgt catggaagat ggtaagggtg aagttacaaa agaagggtgtg
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1561 cattccaaag gaatcattta cagggacctc aagccagaaa atctcatcct agatcaccga
1621 ggttatgcc aactggttga ttttggcttt gcaaagaaaa taggatttgg aaagaaaaca
    
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1681 tggacttttt gtgggactcc agagtatgta gccccagaga tcatcctgaa caaaggccat
1741 gacatttcag ccgactactg gtcactggga atcctaattg atgaactcct gactggcagc
1801 ccacctttct caggcccaga tcctatgaaa acctataaca tcatattgag ggggattgac
1861 atgatagaat ttccaaagaa gattgccaaa aatgctgcta atttaattaa aaaactatgc
1921 aggacaatc catcagaaag attagggaa ttgaaaaatg gagtaaaaga cattcaaaag
1981 cacaaatggt ttgaggcctt taactgggaa ggcttaagaa aaggtacctt gacacctcct
2041 ataataccaa gtgttgcac acccacagac acaagtaatt ttgacagttt ccctgaggac
2101 aacgatgaac caccacctga tgacaactca ggatgggata tagacttcta a
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Reviewed and approved by site quality representative.

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