

Certificate of Analysis

PKD2, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-506, 14-506-K, 14-506M

Parent Lot # 33189U

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, human PKD2, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺-NTA agarose. Purity 52.3% by SDS-PAGE and Coomassie blue staining. MW = 100kDa.

Specific Activity (Parent lot# 33189U): 215U/mg, where one unit of PKD2, active activity is defined as 1nmol phosphate incorporated into 30μM (KKLNRTLVA) per minute at 30°C with a final ATP concentration of 100μM.

Formulation: 1.004mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

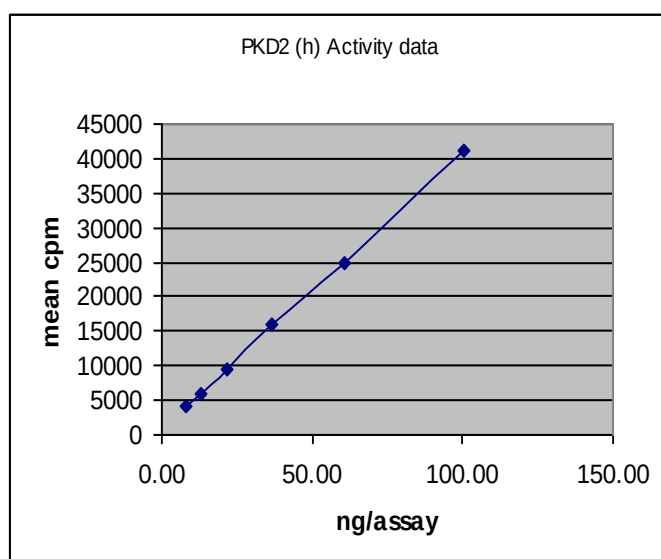
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

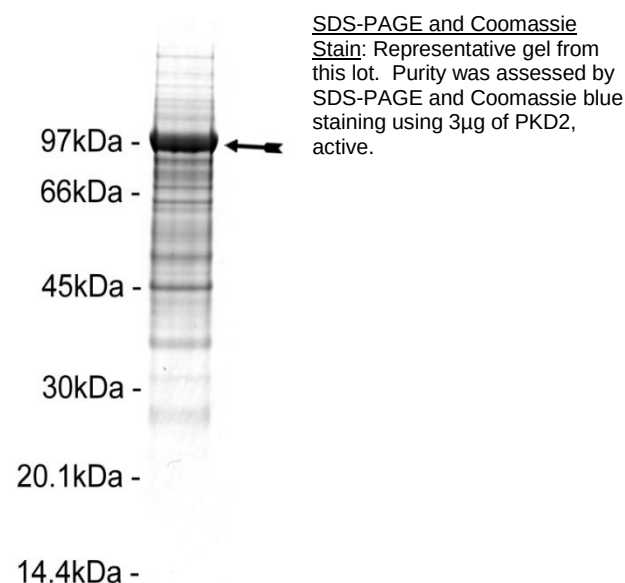
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 8–36ng of this lot of enzyme phosphorylated 30μM (KKLNRTLVA) 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 PKD2 with the translated sequence listed on page three.



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Kinase Assay Protocol

Stock Solutions:

1. **10 x Reaction Buffer:** 200mM HEPES/NaOH pH7.4, 0.3% Triton X-100.
2. **(KKLNRTLSVA):** Use at a final assay concentration of 30 μ M. Prepare a 300 μ M stock. Add 2.5 μ l of stock per assay point.
3. **PKD2, active:** Dilute with 20mM HEPES/NaOH pH7.4, 0.03% Triton X-100. Use 8–36ng per assay point.
4. **[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 (96 well plate format):

1. Add 2.5 μ l of 10 x reaction buffer per assay to wells.
2. Add 2.5 μ l of **(KKLNRTLSVA)**.
3. Add **2.5 μ l (8–36ng) PKD2, active**.
4. Add 7.5 μ l dH₂O.
5. Add 10 μ l of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
8. Transfer a 10 μ l 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 1 μ l of 30% phosphoric acid.

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PKD2 Sequence Information

<u>Protein</u>	Human PKD2
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M29 of the recombinant protein is equivalent to M1 of human PKD2
<u>Accession number</u>	GenBank NM_016457

Recombinant PKD2 amino acid sequence:

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1 MSYYHHHHH DYDIPTTENL YFQGAMGSMA TAPSYAGLP GSPGPGSPPP PGGLELQSP
61 PLLPQIPAPG SGVSFHIQIG LTREFVLLPA ASELAHVKQL ACSIVDQKFP ECGFYGLYDK
121 ILLFKHDPTS ANLLQLVRSS GDIQEGDLVE VVLSASATFE DFQIRPHALT VHSYRAPAFC
181 DHCGEMLFGL VRQGLKCDGC GLNYHKRCFA SIPNNCSGAR KRRLSSTSLA SGHSVRLGTS
241 ESLPCTAEEL SRSTTELLPR RPPSSSSSSS ASSYTGRPIE LDKMLLSKVK VPHTFLIHSY
301 TRPTVCQACK KLLKGLFRQG LQCKDCKFNC HKRCATRVPN DCLGEALING DVPMEETDF
361 SEADKSALMD EEDSGVIPG SHSENALHAS EEEEGEGGKA QSSLGYIPLM RVVQSVRHTT
421 RKSSTTLREG WVVHYSNKDT LRKRHYWRLD CKCITLFQNN TTNRYYKEIP LSEILTVESA
481 QNFSLVPPGT NPHCFEIVTA NATYFVGEMP GGTPGGPSGQ GAEAARGWET AIRQALMPVI
541 LQDAPSAPGH APHRQASLSI SVSNSQIQEN VDIATVYQIF PDEVLGSGQF GVVYGGKHRK
601 TGRDVAVKVI DKLRFPTKQE SQLRNEVAIL QSLRHPGIVN LECMFETPEK VFVVMKHLHG
661 DMLEMILSSE KGRLPRLTK FLITQILVAL RHLHFKNIVH CDLKPENVLL ASADPFPQVK
721 LCDFGFARII GEKSFRRSVV GTPAYLAPEV LLNQGYNRS LDMWSVGVIMY VSLSGTFPFN
781 EDEDINDQIQ NAAFMYASP WSHISAGAI LNNLLQVKM RKRYSDVKSL SHPWLQEYQT
841 WLDLRELEGG MGERYITHES DDARWEQFAA EHPLPGSGLP TDRDLGGACP PQDHDMQGLA
901 ERISVL

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Recombinant PKD2 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
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121 ggctctcccg ggccgggggtc tcctccggcc cccggcgggc tagagctgca gtcgccgcca
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1441 cagaacttca gccttgtgcc gccgggcacc aaccacact gctttgagat cgtcactgcc
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1561 ggggctgagg ccgcccgggg ctgggagaca gccatccgcc aggccctgat gcccgtcatc
1621 cttcaggacg caccagcgc cccaggccac gcgccccaca gacaagcttc tctgagcatc
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2701 gagcgatca gtgttctctg a

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