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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 Ni^{2+}-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 activity is defined as 1nmol phosphate incorporated into 30µM (KKLNRTLSVA) 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.

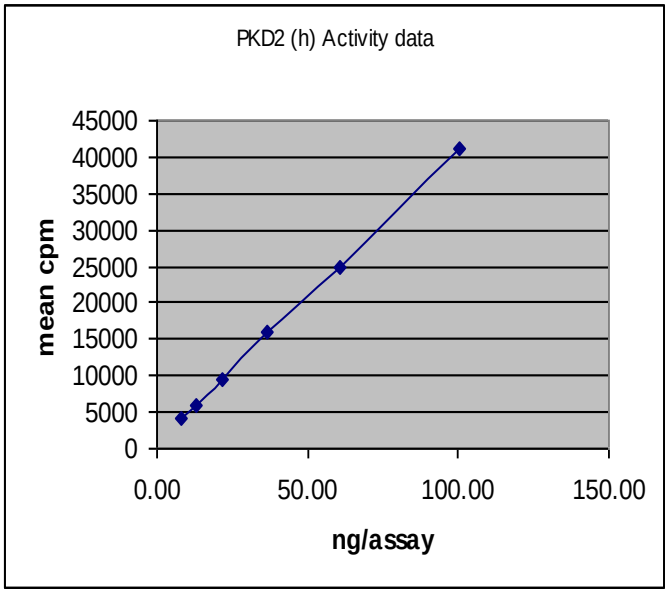
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 6 months from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled microcentrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

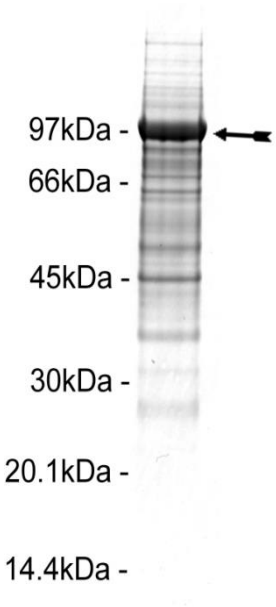
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 8–36ng of this lot of enzyme phosphorylated 30µM (KKLNRTLSVA) 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.



SDS-PAGE and Coomassie Stain: Representative gel from this lot. Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of PKD2.

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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 30µM. Prepare a 300µM stock. Add 2.5µl 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-^{33}P]ATP:** 2.5 x magnesium acetate/[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 2.5µl of 10 x reaction buffer per assay to wells.
2. Add 2.5µl of **(KKLNRTLSVA)**.
3. Add **2.5µl (8–36ng) PKD2, active**.
4. Add 7.5µl dH_2O.
5. Add 10µl of diluted [γ-^{33}P]ATP mixture.
6. Incubate for 10 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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PKD2 Sequence Information

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

Recombinant PKD2 amino acid sequence:

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   1 MSYYHHHHHH DYDIPTTENL YFQGAMGSMA TAPSYPAGLP GSPGPGSPPP PGGLELQSPP
  61 PLLPQIPAPG SGVSFHIQIG LTREFVLLPA ASELAHVKQL ACSIVDQKFP ECGFYGLYDK
 121 ILLFKHDPTS ANLLQLVRSS GDIQEGDLVE VVLSASATFE DFQIRPHALT VHSYRAPAFC
 181 DHCGEMLFGL VRQGLKCDGC GLNYHKRCAF SIPNNCSGAR KRRLSSTSLA SGHSVRLGTS
 241 ESLPCTAEEL SRSTTELLPR RPPSSSSSSS ASSYTGRPIE LDKMLLSKVK VPHTFLIHSY
 301 TRPTVCQACK KLLKGLFRQG LQCKDCKFNC HKRCATRVPN DCLGEALING DVPMEEATDF
 361 SEADKSALMD ESEDSGVIPG 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 VFVVMEKLHG
 661 DMLEMILSSE KGRLPERLTK FLITQILVAL RHLHFKNIVH CDLKPENVLL ASADPFPQVK
 721 LCDFGFARII GEKSFRRSVV GTPAYLAPEV LLNQGYNRSL DMWSVGVIMY VSLSGTFPFN
 781 EDEDINDQIQ NAAFMYPASP WSHISAGAID LINNLLQVKM RKRYSVDKSL SHPWLQEYQT
 841 WLDLRELEGK 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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 541 gatcactgcg gggagatgct cttcggccta gtgcgccagg gcctcaagtg cgatggctgc
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2701 gagcgcatca gtgttctctg a
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Reviewed and approved by site quality representative.