

Certificate of Analysis

PDHK4, active
(Recombinant enzyme expressed in Sf21 insect cells)
Item # 15-024, 15-024-K, 15-024M
Parent Lot # 191200

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 GST-tagged, recombinant, human PDHK4 full length, expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose.
Purity 80% by SDS-PAGE and Coomassie blue staining. MW = 74 kDa.

Specific Activity (Parent lot# 191200): 11 U/mg, where one unit of PDHK4 activity is defined as 1 nmol phosphate incorporated into 250 μ M KKKYHGHSMSPDGPVSYRT per minute at 30°C with a final ATP concentration of 100 μ M.

Formulation: 2.50 mg/ml of enzyme in 50 mM Tris/HCl pH 8.0, 4 mM glutathione, 266 mM NaCl, 0.09 mM EGTA, 0.03% Brij-35, 270 mM sucrose, 0.9 mM benzamidine, 0.2 mM PMSF, 0.09% 2-mercaptoethanol. Frozen solution.

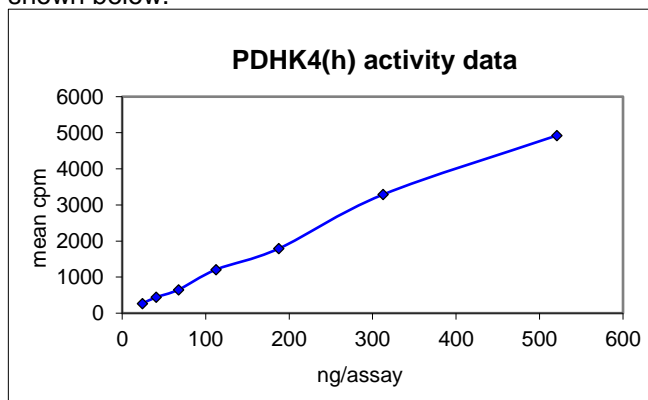
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

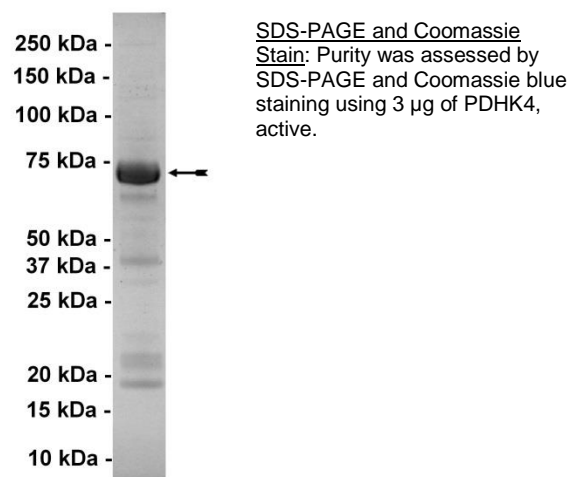
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 24.3–521 ng of this lot of enzyme phosphorylated 250 μ M KKKYHGHSMSPDGPVSYRT 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 PDHK4 with the translated sequence listed on page three.



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Kinase Assay Protocol

Stock Solutions:

- 1. 5 x Reaction Buffer:** 40 mM MOPS/NaOH pH 7.0, 1 mM EDTA.
- 2. KKKYHGHSMSPGVSRYT:** Use at a final assay concentration of 250 μ M. Prepare a 2.5 mM stock and add 2.5 μ l of stock per assay point.
- 3. PDHK4, active:** Dilute with 20 mM MOPS/NaOH pH 7.0, 1 mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1 mg/ml BSA. Use 24.3–521 ng per assay point.
- 4. [γ -³³P]ATP:** 2.5 x MgAc/[γ -³³P]ATP cocktail: 25 mM MgAc and 0.25 mM ATP to which is added [γ -³³P]ATP (specific activity approximately 500 – 800 cpm/pmol as required).

Assay Procedure (96 well plate format):

1. Add 5.0 μ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 μ l of KKKYHGHSMSPGVSRYT
3. Add **2.5 μ l (24.3–521 ng) PDHK4, active.**
4. Add 5.0 μ l of 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 75 mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4 ml 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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PDHK4, active Sequence Information

<u>Protein</u>	Human PDHK4
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	M236 of the recombinant protein is equivalent to M1 of human PDHK4
<u>Accession number</u>	GenBank NM_002612.2

Recombinant PDHK4 amino acid sequence:

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1 MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
61 GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAIPOID KYLKSSKYIA WPLQGWOATF GGGDHPPKSD LVPRGSKEFK GLRRQMKAAAR
241 FVLRASAGSLN GAGLVPREVE HFSRYSPLSPL SMKQLLDFGS ENACERTSFA FLRQELPVRL
301 ANILKEIDIL PTQLVNTSSV QLVKSWYIQS LMDLVEFHEK SPDDQKALSD FVDTLIKVRN
361 RHHNVVPTMA QGIIEYKDAC TVDPVTNQNL QYFLDRFYMN RISTRMLMNQ HILIFSDSQT
421 GNPSHIGSID PNCVVAVVQ DAFECRMLC DQYYLSSPEL KLTQVNGKFP DQPIHIVYVP
481 SHLHHMLFEL FKNAMRATVE HQENQPSLTP IEVIVVLGKE DLTIKISDRG GGVPLRIIDR
541 LFSYTYSTAP TPVMDNSRNA PLAGFGYGLP ISRLYAKYFQ GDLNLYSLSG YGTDAIYYLK
601 ALSSESIEKL PVFNKSAFKH YQMSSEADDW CIPSREPKNL AKEVAM

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Recombinant PDHK4 nucleotide sequence:

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1 atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaaccacac tcgacttctt
61 ttggaatata ttgaagaaaa atatgaagag catttgtatg agcgcgatga aggtgataaa
121 tggcgaaca aaaagtttga attgggtttg gagtttccca atcttcctta ttatattgat
181 ggtgatgta aattaacaca gtctatggcc atcatacggtt atatagctga caagcacaac
241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcgggtttg
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1501 caccaggaaa atcagccttc ctttacacca atagaggtta ttgttgtctt gggaaaagaa
1561 gaccttacca ttaagatttc agacagagga ggtggtgttc ccctgagaat tattgaccgc
1621 ctcttttagt atacatact cactgcacca acgcctgtga tggataatcc cgggaatgct

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1681 cctttggctg gttttggtta cggcttgcca atttctcgtc tgtatgcaa gtactttcaa
1741 ggagatctga atctctactc tttatcagga tatggaacag atgctatcat ctacttaaag
1801 gctttgtctt ctgagtctat agaaaaactt ccagttttta acaagtcagc cttcaaacad
1861 tatcagatga gctctgaggc tgatgactgg tgtatcccaa gcaggggaacc aaagaacctg
1921 gcaaaagaag tggccatgtg a
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Reviewed and approved by site quality representative.

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