

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 vialing runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialing 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 = 74kDa.

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

Formulation: 2.50mg/ml of enzyme in 50mM Tris/HCl pH8.0, 4mM glutathione, 266mM NaCl, 0.09mM EGTA, 0.03% Brij-35, 270mM sucrose, 0.9mM benzamidine, 0.2mM PMSF, 0.09% 2-mercaptoethanol. Frozen solution.

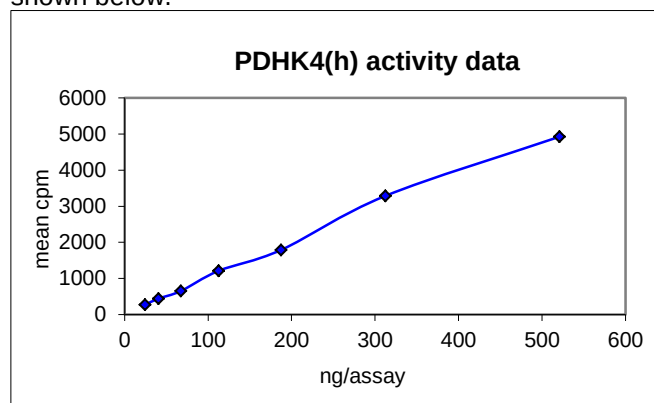
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

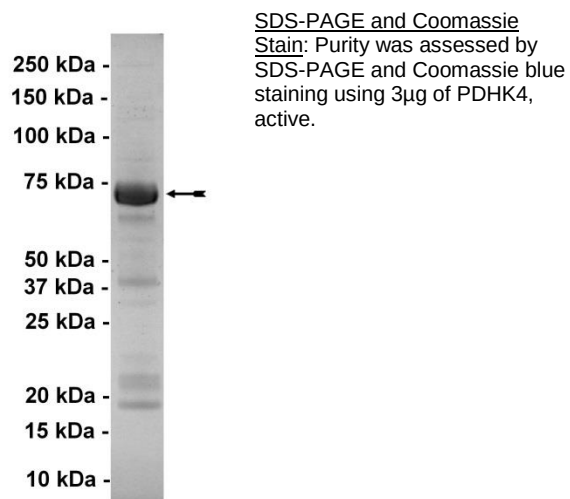
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 24.3–520.8ng of this lot of enzyme phosphorylated 250μM KKKYHGHSMSPGVSRYT 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:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
- 2. KKKYHGHSMSPGVSYRT:** Use at a final assay concentration of 250µM. Prepare a 2.5mM stock and add 2.5µl of stock per assay point.
- 3. PDHK4, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 24.3–520.8ng per assay point.
- 4. [γ -³³P]ATP:** 2.5 x MgAc/[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 5.0µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of KKKYHGHSMSPGVSYRT
3. Add **2.5µl (24.3–520.8ng) PDHK4, active.**
4. Add 5.0µl of dH₂O.
5. Add 10µl of diluted [γ -³³P]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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PDHK4, active Sequence Information

Protein	Human PDHK4
Tags	N-terminal GST
Native sequence	M236 of the recombinant protein is equivalent to M1 of human PDHK4
Accession number	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  KRIEAIQID  KYLKSSKYIA  WPLQGWQATF  GGGDHPPKSD  LVPRGSKEFK  GLRRQMKAAR
241  FVLRSAAGSLN  GAGLVPREVE  HFSRYSPL  SMKQLLDFGS  ENACERTSFA  FLRQELPVRL
301  ANILKEIDIL  PTQLVNTSSV  QLVKSWYIQS  LMDLVEFHEK  SPDDQKALSD  FVDTLIKVRN
361  RHHNVVPTMA  QGIIEYKDAC  TVDPVTNQNL  QYFLDRFYMN  RISTRMLMNQ  HILIFSDSQT
421  GNPSHIGSID  PNCDDVAVVQ  DAFECRMLC  DQYYLSSPEL  KLTQVNGKFP  DQPIHIVYVP
481  SHLHHMLFEL  FKNAMRATVE  HQENQPSLTP  IEVIVVLGKE  DLTIKISDRG  GGVPLRIIDR
541  LFSYTYSTAP  TPVMDNSRNA  PLAGFGYGLP  ISRLYAKYFQ  GDLNLYSLSG  YGTDAIYLYK
601  ALSSESIEKL  PVFNKSAFKH  YQMSSEADDW  CIPSREPKNL  AKEVAM

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Recombinant PDHK4 nucleotide sequence:

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1  atgtccccta  tactaggtta  ttggaaaatt  aagggccttg  tgcaaccac  tgcacttctt
61  ttggaatatt  ttgaagaaaa  atatgaagag  catttgatat  agcgcgatga  aggtgataaa
121  tggcgaaaca  aaaagtttga  attgggtttg  gagtttccca  atcttcctta  ttatattgat
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1561  gaccttacca  ttaagatttc  agacagagga  ggtggtgttc  ccctgagaat  tattgaccgc
1621  ctcttttagtt  atacatactc  cactgcacca  acgctgtgta  tggataattc  ccggaatgct

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1801 gctttgtctt ctgagtctat agaaaaactt ccagttttta acaagtcagc cttcaaacad
1861 tatcagatga gctctgaggc tgatgactgg tgtatcccaa gcagggaacc aaagaacctg
1921 gcaaaagaag tggccatgtg a
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