

Product Information

**PDHK3, active, GST-tagged, human
PRECISIO® Kinase
recombinant, expressed in Sf9 cells**

Catalog Number **SRP5249**

Storage Temperature -70°C

Synonym: PDK3

Product Description

PDHK3 or Pyruvate Dehydrogenase Kinase 3 is a member of the PDHK family that phosphorylates and inactivates Pyruvate Dehydrogenase (PDH).¹ PDH is a mitochondrial multienzyme complex that catalyzes the oxidative decarboxylation of pyruvate and is one of the major enzymes responsible for the regulation of homeostasis of carbohydrate fuels in mammals. HIF-1 induces PDHK3 expression leading to inhibition of mitochondrial respiration.² Forced expression of PDHK3 in cancer cells results in increased lactic acid accumulation and drugs resistance; whereas, knocking down PDHK3 inhibits hypoxia-induced cytoplasmic glycolysis and cell survival.

Recombinant full-length human PDHK3 was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The PDHK3 gene accession number is NM_005391. Recombinant protein stored in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 mM glutathione, 0.1 mM EDTA, 0.25 mM DTT, 0.1 mM PMSF, and 25% glycerol.

Molecular mass: ~ 69 kDa

Purity: 70–95% (SDS-PAGE, see Figure 1)

Specific Activity: 10.2–13.8 nmole/min/mg
(see Figure 2)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The product ships on dry ice and storage at -70°C is recommended. After opening, aliquot into smaller quantities and store at -70°C . Avoid repeated handling and multiple freeze/thaw cycles.

Figure 1.
SDS-PAGE Gel of Typical Lot
70–95% (densitometry)

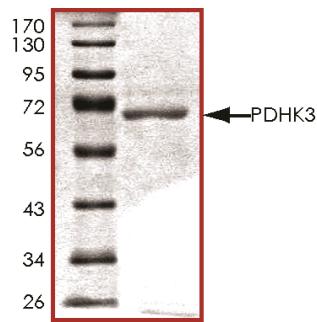
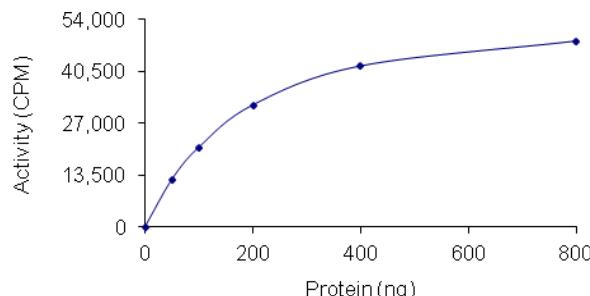


Figure 2.
Specific Activity of Typical Lot
10.2–13.8 nmole/min/mg



Procedure

Preparation Instructions

Kinase Assay Buffer – 25 mM MOPS, pH 7.2, 12.5 mM glycerol 2-phosphate, 25 mM MgCl₂, 5 mM EGTA, and 2 mM EDTA. Just prior to use, add DTT to a final concentration of 0.25 mM.

Kinase Dilution Buffer – Dilute the Kinase Assay Buffer 5-fold with a 50 ng/ μl BSA solution.

Kinase Solution – Dilute the active PDHK3 (0.1 μ g/ μ l) with Kinase Dilution Buffer to the desired concentration.

Note: The lot-specific specific activity plot may be used as a guideline (see Figure 2). It is recommended the researcher perform a serial dilution of active PDHK3 kinase for optimal results.

10 mM ATP Stock Solution – Dissolve 55 mg of ATP in 10 ml of Kinase Assay Buffer. Store in 200 μ l aliquots at –20 °C.

γ -³³P-ATP Assay Cocktail (250 μ M) – Combine 5.75 ml of Kinase Assay Buffer, 150 μ l of 10 mM ATP Stock Solution, 100 μ l of γ -³³P-ATP (1 mCi/100 μ l). Store in 1 ml aliquots at –20 °C.

Substrate Solution – PDHA1 protein substrate prepared in 50 mM sodium phosphate, pH 7.0, 300 mM NaCl, 150 mM imidazole, 0.25 mM DTT, 25% glycerol to a final concentration of 0.2 μ g/ μ l.

1% phosphoric acid solution – Dilute 10 ml of concentrated phosphoric acid to a final volume of 1 L with water.

Kinase Assay

This assay involves the use of the ³³P radioisotope. All institutional guidelines regarding the use of radioisotopes should be followed.

1. Thaw the active PDHK3, Kinase Assay Buffer, Substrate Solution, and Kinase Dilution Buffer on ice. The γ -³³P-ATP Assay Cocktail may be thawed at room temperature.
2. In a pre-cooled microcentrifuge tube, add the following solutions to a volume of 20 μ l:
10 μ l of Kinase Solution
10 μ l of Substrate Solution
3. Set up a blank control as outlined in step 2, substituting 10 μ l of cold water (4 °C) for the Substrate Solution.
4. Initiate each reaction with the addition of 5 μ l of the γ -³³P-ATP Assay Cocktail, bringing the final reaction volume to 25 μ l. Incubate the mixture in a water bath at 30 °C for 15 minutes.
5. After the 15 minute incubation, stop the reaction by spotting 20 μ l of the reaction mixture onto an individually pre-cut strip of phosphocellulose P81 paper.

6. Air dry the pre-cut P81 strip and sequentially wash in the 1% phosphoric acid solution with constant gentle stirring. It is recommended the strips be washed a total of 3 times of ~10 minutes each.
7. Set up a radioactive control to measure the total γ -³³P-ATP counts introduced into the reaction. Spot 5 μ l of the γ -³³P-ATP Assay Cocktail on a pre-cut P81 strip. Dry the sample for 2 minutes and read the counts. Do not wash this sample.
8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
9. Determine the corrected cpm by subtracting the blank control value (see step 3) from each sample and calculate the kinase specific activity

Calculations:

1. Specific Radioactivity (SR) of ATP (cpm/nmole)

$$SR = \frac{\text{cpm of } 5 \mu\text{l of } \gamma\text{-}^{33}\text{P-ATP Assay Cocktail}}{\text{nmole of ATP}}$$

cpm – value from control (step 7)

nmole – 1.25 nmole (5 μ l of 250 μ M ATP Assay Cocktail)

2. Specific Kinase Activity (SA) (nmole/min/mg)

$$\text{nmole/min/mg} = \frac{\Delta\text{cpm} \times (25/20)}{SR \times E \times T}$$

SR = specific radioactivity of the ATP (cpm/nmole ATP)

Δ cpm = cpm of the sample – cpm of the blank (step 3)

25 = total reaction volume

20 = spot volume

T = reaction time (minutes)

E = amount of enzyme (mg)

References

1. Gudi, R. et al., Diversity of the pyruvate dehydrogenase kinase gene family in humans. *J. Biol. Chem.*, **270**, 28989-28994 (1995).
2. Lu, C.W. et al., Induction of pyruvate dehydrogenase kinase-3 by hypoxia-inducible factor-1 promotes metabolic switch and drug resistance. *J Biol Chem.*, **283(42)**, 28106-14 (2008).

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