

## Product Information

**PDHK1, active, GST-tagged, human  
PRECISIO® Kinase  
recombinant, expressed in Sf9 cells**

Catalog Number **SRP5247**  
Storage Temperature  $-70^{\circ}\text{C}$

Synonym: PDK1

### Product Description

PDHK1 or Pyruvate Dehydrogenase Kinase 1 is a member of the PDHK family that phosphorylates and inactivates the Pyruvate Dehydrogenase (PDH).<sup>1</sup> 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. PDHK1 is an important regulator of PDH in clonal  $\beta$ -cells, and the activity of PDHK1 and PDH are important for efficient metabolic coupling. Maintaining low PDHK1 expression/activity and keeping PDH in a dephosphorylated and active state, is important for  $\beta$ -cells to achieve the metabolic flux rates necessary for maximal glucose-stimulated insulin secretion.<sup>2</sup>

Recombinant full-length human PDHK1 was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The PDHK1 gene accession number is NM\_002610. 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: ~70 kDa

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

Specific Activity: 1.4–2 nmole/min/mg (see Figure 2)

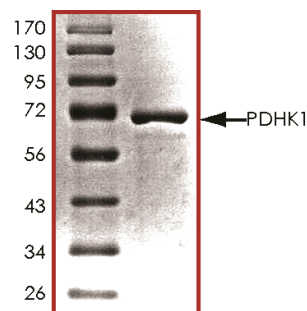
### Precautions and Disclaimer

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.

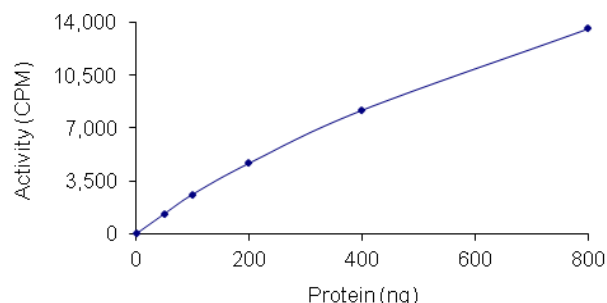
### Storage/Stability

The product ships on dry ice and storage at  $-70^{\circ}\text{C}$  is recommended. After opening, aliquot into smaller quantities and store at  $-70^{\circ}\text{C}$ . 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  
1.4–2 nmole/min/mg



### Procedure

#### Preparation Instructions

Kinase Assay Buffer – 25 mM MOPS, pH 7.2, 12.5 mM glycerol 2-phosphate, 25 mM  $\text{MgCl}_2$ , 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/ $\mu\text{L}$  BSA solution.

Kinase Solution – Dilute the active SRP5247 (0.1 µg/µl) 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 SRP5247 kinase for optimal results.

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

γ-<sup>33</sup>P-ATP Assay Cocktail (250 µM) – Combine 5.75 ml of Kinase Assay Buffer, 150 µl of 10 mM ATP Stock Solution, 100 µl of γ-<sup>33</sup>P-ATP (1 mCi/100 µl). 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, and 25% glycerol to a final concentration of 0.2 µg/µl.

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 <sup>33</sup>P radioisotope. All institutional guidelines regarding the use of radioisotopes should be followed.

1. Thaw the active SRP5247, Kinase Assay Buffer, Substrate Solution, and Kinase Dilution Buffer on ice. The γ-<sup>33</sup>P-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 µl:  
10 µl of Kinase Solution  
10 µl of Substrate Solution
3. Set up a blank control as outlined in step 2, substituting 10 µl of cold water (4 °C) for the Substrate Solution.
4. Initiate each reaction with the addition of 5 µl of the γ-<sup>33</sup>P-ATP Assay Cocktail, bringing the final reaction volume to 25 µl. 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 µl 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-<sup>33</sup>P-ATP counts introduced into the reaction. Spot 5 µl of the γ-<sup>33</sup>P-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 µl of 250 µM 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)

Δcpm = 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. Krus, U. et al., Pyruvate dehydrogenase kinase 1 controls mitochondrial metabolism and insulin secretion in INS-1 832/13 clonal beta-cells. Biochem. J., **429**(1), 205-13 (2010).

PRECISIO is a registered trademark of Sigma-Aldrich Co. LLC.

RC,MAM 10/11-1