Recombinant Human PCK1/PEPCK1 His-tag

Catalog Number: 11818-PK

DESCRIPTION

Source	*E. coli*-derived human PCK1 protein Pro2-Met622, with an N-terminal Met and 6-His tag Accession # P35558.3
N-terminal Sequence Analysis	Met
Predicted Molecular Mass	70 kDa

SPECIFICATIONS

SDS-PAGE	61-68 kDa, under reducing conditions.
Activity	Measured by its ability to transfer phosphate from adenosine triphosphate (ATP) to Oxaloacetic Acid. The specific activity is >200 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<0.10 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Supplied as a 0.2 µm filtered solution in Tris, NaCl and TCEP. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 20 mM $MgCl_2$, 5 mM $MnCl_2$, 0.1 mg/mL BSA, pH 7.5
- Recombinant Human PCK1/PEPCK1 His-tag (rhPCK1) (Catalog # 11818-PK)
- Oxaloacetic Acid, 100 mM stock in deionized water
- Adenosine triphosphate (ATP), 10 mM stock in deionized water
- ADP-Glo™ Kinase Assay Kit (Promega)
- White 96-well Plate
- Plate Reader with Luminescence Read Capability

Assay

1. Dilute rhPCK1 to 1 µg/mL in Assay Buffer.
2. Prepare Substrate Mixture containing 200 µM ATP and 10 mM Oxaloacetic Acid in Assay Buffer.
3. Combine equal volumes of 1 µg/mL rhPCK1 and Substrate Mixture. Create a Substrate Control by replacing enzyme with Assay Buffer.
4. Incubate at room temperature for 40 minutes in the dark.
5. After incubation, transfer 10 µL of each reaction to wells of a white plate.
6. Terminate the reaction and deplete the remaining ATP by adding 10 µL of ADP-Glo™ Reagent (supplied in kit) to all wells.
7. Incubate at room temperature for 40 minutes in the dark.
8. Add 20 µL of Kinase Detection Reagent (supplied in kit) to all wells.
9. Incubate at room temperature for 30 minutes in the dark.
10. Read plate in Luminescence endpoint mode.
11. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted Luminescence* (RLU) x Conversion Factor** (pmol/RLU)}}{\text{Incubation time (min) x amount of enzyme (µg)}}$$

*Adjusted for Substrate Control

**Derived from ADP-Glo™ Kinase Assay Kit protocol (Promega)

Final Assay Conditions

Per Reaction:

- rhPCK1: 0.5 µg/mL
- ATP:100 µM
- Oxaloacetic Acid: 5 mM

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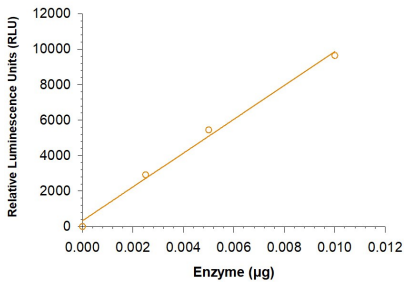
PREPARATION AND STORAGE

Shipping	The product is shipped with dry ice or equivalent. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 6 months from date of receipt, -20 to -70 °C as supplied. • 3 months, -20 to -70 °C under sterile conditions after opening.

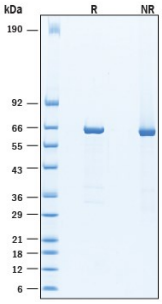
DATA

Enzyme Activity



Recombinant Human PCK1/PEPCK1 His-tag Enzyme Activity. Recombinant Human PCK1/PEPCK1 His-tag Protein (Catalog # 11818-PK) is measured by its ability to transfer phosphate from adenosine triphosphate (ATP) to Oxaloacetic Acid.

SDS-Page



Recombinant Human PCK1/PEPCK1 His-tag SDS-PAGE. 2 µg/lane of Recombinant Human PCK1/PEPCK1 His-tag (Catalog # 11818-PK) was resolved with SDS-PAGE under reducing (R) condition and visualized by Coomassie® Blue staining, showing bands at 61-68 kDa, under reducing conditions.

BACKGROUND

Recombinant human cytosolic Phosphoenolpyruvate carboxykinase (PEPCK1), also known as Serine protein kinase PCK1, is the cytosolic form of two highly conserved rate-limiting mammalian PEPCK isoenzymes that catalyze the generation of phosphoenolpyruvate (PEP) from oxaloacetate (OAA) in gluconeogenesis. While both forms are structurally and functionally similar, they have differing activity and expression in tissues which leads to distinct biological function (1). While PEPCK2 is widely expressed and induced by stress, PEPCK1 is highly expressed in the liver, kidney, gastrointestinal tract and adipose tissue (1). PEPCK1 is a monomeric protein that has a conserved functional active site and contains carboxylate group-binding motifs for PEP and OAA guanine- and ribose-binding sites that form an unusual pocket nucleotide binding site for GTP nucleotides, and a Mn-binding site (2-4). PEPCK1 can be acetylated to regulate its activity (1, 5). Due to the critical nature of PEPCK1 as a rate-limiting enzyme in gluconeogenesis, misregulation of the enzyme is problematic. Mutations in the gene can lead to autosomal recessive metabolic disorders known as phosphoenolpyruvate carboxykinase deficiency that can be fatal (1, 6, 7), downregulation can lead to metabolic associated fatty liver disease (MAFLD) (8), and overactivation of PEPCK activity has been linked to diabetes (9). Given its role as a control point in metabolic gluconeogenesis and its responsiveness to glucocorticoids and insulin, PEPCK1 is of interest for a potential therapeutic target in diabetes and obesity (1, 10, 11). In addition, given its role in metabolic regulation directly or via kinase sterol regulatory element-binding protein (srebp) activation to regulate the transcription of lipogenesis genes, altered PEPCK1 function has been reported in many cancers including liver, non-small cell lung cancer (NSCLC), and esophageal melanoma (1, 12-14). Expression changes of PEPCK1 were also linked to prognosis in several cancers including lung, liver, pancreatic, esophageal and renal carcinoma (13-18). PEPCK1 is downregulated in many tumors of gluconeogenic organs (HCC and RCC) and has anti-tumor effects while PEPCK1 is upregulated and oncogenic in tumors originating from non-gluconeogenic organs (CRC, lung cancer, melanoma, breast cancer, pancreatic cancer, and gastric cancer) making PEPCK1 a target for broad therapeutic application in cancer treatments (4, 15-18).

References:

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