Recombinant Human PGK1

Catalog Number: 5455-PK

DESCRIPTION

Source	*Spodoptera frugiperda*, *Sf* 21 (baculovirus)-derived Met1-Ile417, with an C-terminal 10-His tag Accession # P00558
N-terminal Sequence Analysis	No results obtained
Predicted Molecular Mass	46 kDa

SPECIFICATIONS

SDS-PAGE	42 kDa, reducing conditions
Activity	Measured by NADH production in a reaction coupled with GAPDH. The specific activity, as measured under the described condtions, is >50,000 pmol/min/µg.
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE under reducing conditions and visualized by silver stain.
Formulation	Supplied as a 0.2 µm filtered solution in Tris, NaCl and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Diluent: deionized water
- Recombinant Human PGK1 (rhPGK1) (Catalog # 5455-PK)
- 50 mM KH_2PO_4, pH 7.0
- 100 mM $MgSO_4$ in deionized water
- 1.0 M Glycine in deionized water
- 50 mM DL-Glyceraldehyde 3-Phosphate (GAP) (Sigma, Catalog # G5251) in deionized water
- 10 mM β-Nicotinamide adenine dinucleotide (β-NAD) (Sigma, Catalog # N6522). Prepare 200 mM stock in deionized water
- 10 mM Adenosine 5'-Diphosphate (ADP) (Sigma, Catalog # A2754). Prepare 200 mM stock in deionized water. Note: ADP degrades to AMP which is an inhibitor of rhPGK1. Be sure to aliquot and store the stock at ≤-20 °C. Prepare fresh when necessary.
- 0.25 µg/µL Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH) (Sigma, Catalog # G5537) in 50% Glycerol
- UV Plate, 96 well (Costar, Catalog # 3635)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Prepare substrate buffer by mixing the following (prepare fresh):
   a. 100 µL 50 mM KH_2PO_4, pH 7.0
   b. 20 µL 50 mM GAP
   c. 30 µL 10 mM β-NAD
   d. 20 µL 10 mM ADP
   e. 50 µL 100 mM $MgSO_4$
   f. 100 µL 1 M Glycine
   g. 20 µL 0.25 µg/µL GAPDH
   h. 160 µL deionized water
   Note: This amount will assay nine wells. If more volume is needed, multiply each component's volume by the same number to get the desired amount.
2. Dilute rhPGK1 to 0.02 ng/µL in deionized water.
3. Load in a 96 well UV plate 50 µL of the substrate buffer, and start the reaction by adding 50 µL of 0.02 ng/µL rhPGK1. Include a blank containing 50 µL of the substrate buffer and 50 µL deionized water.
4. Read at 339 nm in kinetic mode for 5 minutes.
5. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{max}^{*} \text{ (OD/min) x well volume (L) x } 10^{12} \text{ pmol/M}}{\text{ext. coeff}^{**} \text{ (M}^{-1}\text{cm}^{-1}\text{) x path corr.}^{***} \text{ (cm) x amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank

**Using the extinction coefficient 6220 $M^{-1}cm^{-1}$

***Using the path correction 0.32 cm

Note: the output of many spectrophotometers is in mOD

Final Assay Conditions

Per Well:

- rhPGK1: 0.001 µg
- Rxn mix: 5 mM KH_2PO_4, pH 7.0, 1 mM GAP, 0.3 mM β-NAD, 0.2 mM ADP, 5 mM MgSO4, 100 mM Glycine, 5 ng/µL GAPDH

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, -70 °C as supplied. ● 3 months, -70 °C under sterile conditions after opening.

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BACKGROUND

Phosphoglycerate kinase-1 (PGK-1) is a glycolytic enzyme that catalyzes the conversion of 1,3-diphosphoglycerate to 3 phosphoglycerate. The gene encoding PGK-1 is X-linked. Mutations of this gene may cause phosphoglycerate kinase deficiency, which is characterized by hemolytic anemia, muscle stiffness and mental retardation (1-3). PGK 1 is induced by oxidative stress through the induction of hypoxia-inducible factor 1a and is a potential biomarker and therapeutic target for cancer (4-7).

References:

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