Recombinant Human Liver Glycogen Phosphorylase His-tag

Catalog Number: 11787-PL

DESCRIPTION

Source	Human embryonic kidney cell, HEK293-derived human PYGL protein Ala2 - Asn847, with an N-terminal Met and 6-His tag Accession # P06737.4
N-terminal Sequence Analysis	Protein identity confirmed by mass spectrometry.
Predicted Molecular Mass	98 kDa

SPECIFICATIONS

SDS-PAGE	85-94 kDa, under reducing conditions.
Activity	Measured by its ability to hydrolyze α-D-Glucose 1 -phosphate. The specific activity is >400 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, Glycerol and TCEP. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM MES, pH 6.0
- Recombinant Human Liver Glycogen Phosphorylase His-tag (rhPYGL) (Catalog # 11787-PL)
- Adenosine monophosphate (AMP), 5 mM stock in deionized water
- Substrate: Glycogen, 20 mg/mL stock in deionized water
- Substrate: α-D-Glucose-1-Phosphate, 10 mM stock in deionized water
- Malachite Green Phosphate Detection Kit (Catalog # DY996)
- Clear 96-well Plate (Catalog # DY990)
- Plate Reader with Absorbance Read Capability

Assay

1. Dilute 1 M Phosphate Standard (supplied in kit) by adding 10 µL of the 1 M Phosphate Standard to 990 µL of Assay Buffer for a 10 mM stock. Continue by adding 10 µL of the 10 mM Phosphate stock to 990 µL of Assay Buffer for a 100 µM stock. This is the first point of the standard curve.
2. Complete the standard curve by performing six one-half serial dilutions of the 100 µM Phosphate stock in Assay Buffer. The standard curve has a range of 0.078 to 5 nmol per well.
3. Load 50 µL of each dilution of the standard curve into a plate. Include a curve blank containing 50 µL of Assay Buffer.
4. Dilute rhPYGL to 3 µg/mL in Assay Buffer.
5. Load 25 µL of 3 µg/mL rhPYGL into empty wells of the same plate as the curve. Include a Control containing 25 µL of Assay Buffer.
6. Create a reaction mixture containing 0.5 mM AMP, 2 mM α-D-Glucose-1-Phosphate and 4 mg/mL Glycogen in Assay Buffer.
7. Add 25 µL of reaction mixture to the wells, excluding the standard curve.
8. Seal plate and incubate at room temperature for 20 minutes.
9. Add 30 µL of the Malachite Green Reagent A to all wells. Mix briefly.
10. Add 100 µL of deionized water to all wells. Mix briefly.
11. Add 30 µL of the Malachite Green Reagent B to all wells. Mix and incubate for 20 minutes at room temperature.
12. Read plate at 620 nm (absorbance) in endpoint mode.
13. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Phosphate released* (nmol) x (1000 pmol/nmol)}}{\text{Incubation time (min) x amount of enzyme (µg)}}$$

*Derived from the phosphate standard curve using linear or 4-parameter fitting and adjusted for Control.

Final Assay Conditions

Per Reaction:

- rhPYGL: 0.075 µg
- AMP: 0.25 mM
- α-D-Glucose-1-Phosphate: 1 mM
- Glycogen: 100 µg

PREPARATION AND STORAGE

Shipping

The product is shipped with polar packs. 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

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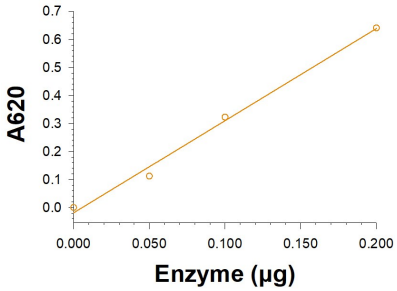
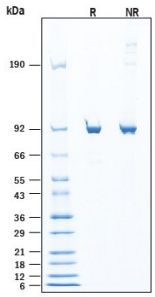
Enzyme Activity



Recombinant Human Liver Glycogen Phosphorylase His-tag Enzyme Activity. Recombinant Human Liver Glycogen Phosphorylase His-tag (Catalog # 11787-PL) is measured by its ability to hydrolyze α-D-Glucose 1-phosphate.

SDS-PAGE



Recombinant Human Liver Glycogen Phosphorylase His-tag SDS-PAGE. 2 µg/lane of Recombinant Human Liver Glycogen Phosphorylase His-tag (Catalog # 11787-PL) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 85-94 kDa, under reducing conditions.

BACKGROUND

Recombinant human liver glycogen phosphorylase (PYGL) is a cytoplasmic liver isoform of glycogen phosphorylase from a family of three mammalian isoforms expressed in liver, muscle, or brain tissue that differ in their activation regulation (1, 2). PYGL catalyzes the cleavage of α-1,4-glycosidic bonds in glycogen to release glucose-1-phosphate and represents the rate-limiting step of hepatic glycogenolysis and a key control point in maintaining blood glucose during fasting (1). In diabetic subjects, glycogenolysis remains an important contributor to hepatic glucose output even when blood glucose levels are high, establishing a potential role for phosphorylase inhibitors in diabetes therapy (3, 4). PYGL is biologically active as a homodimer where each 846-residue monomer comprises N- and C-terminal domains that form the catalytic clefts at the homodimeric interface (4). The C-terminal domain contains a cofactor binding site for covalently bound pyridoxal cofactor. The N-terminal domain contains a glycogen storage allosteric site and several additional regulatory sites such as the phosphorylation site that regulates conformation of loops that control access of the substrate to the active site and an AMP-activator site that stabilizes the allosterically regulated active conformation when AMP is bound and the inactive conformation when other phosphoryl ligands are bound (1, 4). The regulation of PYGL activity via multiple allosteric sites presents opportunities for druggable pocket exploitation with small-molecule ligands and manipulation of phosphorylation state by phosphorylase kinase and protein phosphatase 1 (1, 4). Given the critical role it plays in gluconeogenesis, PYGL is implicated as a metabolism-related oncogenic biomarker and potential target of interest in several cancers including glioblastoma, renal cell carcinoma, pancreatic, lung, and head and neck squamous cell carcinoma as its upregulation is correlated with poor survival due to its enhancement of malignancy, role in signaling through glycogen level regulation including O-GlcNAcylation, and contributions to chemotherapy resistance (2, 5-9). Targeting PYGL could be an effective therapeutic strategy in several cancers and also enhance the efficacy of immunotherapies due to its role in regulation of metabolism and immune suppression (2, 6, 8, 9). Finally, pathogenic splice-site variants and highly conserved missense variants of PYGL that result in deficiency cause an autosomal recessive disorder of metabolism known as Glycogen storage disease type VI (GSD-VI) or Hers disease, that can lead to liver cirrhosis and other complications (10, 11).

References:

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