Recombinant Human Muscle Glycogen Phosphorylase His-tag

Catalog Number: 11786-PM

DESCRIPTION

Source	Human embryonic kidney cell, HEK293-derived human Glycogen phosphorylase, muscle form protein Ser2-Ile842, with an N-terminal Met and 6-His tag Accession # P11217.6
N-terminal Sequence Analysis	Protein identity confirmed by mass spectrometry.
Predicted Molecular Mass	98 kDa

SPECIFICATIONS

SDS-PAGE	86-96 kDa, under reducing conditions
Activity	Measured by its ability to hydrolyze α-D-Glucose 1-phosphate. The specific activity is >3000 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, TCEP and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, pH 7.0
- Recombinant Human Muscle Glycogen Phosphorylase His-tag (rhPYGM) (Catalog # 11786-PM)
- Adenosine monophosphate (AMP), 5 mM stock in deionized water
- Substrate: α-D-Glucose-1-Phosphate, 10 mM stock in deionized water
- Substrate: Glycogen, 20 mg/mL 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 rhPYGM to 0.5 µg/mL in Assay Buffer.
5. Load 25 µL of 0.5 µg/mL rhPYGM 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 1 mM AMP, 1 mM α-D-Glucose-1-Phosphate and 2 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:

- rhPYGM: 0.0125 µg
- AMP: 0.5 mM
- α-D-Glucose-1-Phosphate: 0.5 mM
- Glycogen: 50 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.

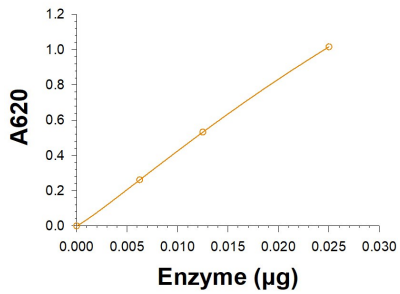
DATA

Bio-Techne®
Global | bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL: 1.612.379.2956
USA | TEL: 800.343.7475 Canada | TEL: 855.668.8722 Europe | Middle East | Africa TEL: +44.0.1235.529449
China | info.cn@bio-techne.com TEL: 400.821.3475

Recombinant Human Muscle Glycogen Phosphorylase His-tag

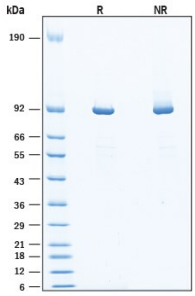
Catalog Number: 11786-PM

Enzyme Activity



Recombinant Human Muscle Glycogen Phosphorylase His-tag Enzyme Activity. Recombinant Human Muscle Glycogen Phosphorylase His-tag (Catalog # 11786-PM) is measured by its ability to hydrolyze α-D-Glucose 1-phosphate.

SDS-PAGE



Recombinant Human Muscle Glycogen Phosphorylase His-tag SDS-PAGE. 2 µg/lane of Recombinant Human Muscle Glycogen Phosphorylase His-tag (Catalog # 11786-PM) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 86-96 kDa, under reducing conditions.

BACKGROUND

Recombinant Human Muscle Glycogen Phosphorylase (PYGM), also known as myophosphorylase, is a cytoplasmic muscle isoform of glycogen phosphorylase (GP) that catalyzes the cleavage of α-1,4-glycosidic bonds in glycogen to release glucose-1-phosphate. PYGM is one of three mammalian isoforms known as liver, muscle, or brain GP that differ in sequence, activation regulation, kinetics, and physiological roles (1-4). PYGM is biologically active as a homodimer where each 842-residue monomer comprises N- and C-terminal domains (1,3). The C-terminal domain contains a cofactor binding site for covalently bound pyridoxal cofactor. The N-terminal domain contains a key phosphorylation site that determines whether the protein is in an active or inactive state; each state is further regulated by binding of AMP, ATP, and glucose-6-phosphate in an allosteric site within the N-terminal domain. The N-terminal domain also contains a glycogen storage site. The muscle isoform PYGM is uniquely responsive to glucose for transition to an inactive state and binds AMP in a cooperative manner unique from the other isoforms. PYGM must be able to quickly respond to acute demands of energy through production of ATP for many biological processes in cells including contraction in muscles (1,3,4) and is responsive to extracellular control through neural and hormonal signals (2). PYGM is expressed in skeletal muscle but also is expressed as a predominant form of glycogen phosphorylase in the nervous system (5-7) and T lymphocytes where it binds the active form of proto-oncogenic RAC1 and leads to T-cell migration and proliferation (4,8,9). Based on regulation through extracellular signaling pathways, PYGM influences cellular processes such as signal transduction, transcription, protein stability, and cell viability through its involvement in insulin and glucagon signaling, the insulin resistance pathway, the hexosamine biosynthetic pathway where it plays a role in dynamic post-translational protein O-GlcNAcylation (7) in addition to glycogen metabolism. PYGM is also implicated and targeted as a metabolism-related oncogenic biomarker that is mis-regulated in many types of cancer including sarcoma, head and neck squamous cell carcinoma, and rectal cancer where it is often correlated with poor survival rate (4, 10-12). Finally, pathogenic dominant and recessive PYGM mutations result in glycogen accumulation and aggregates in a dominant mutation and over 200 described mutations result in autosomal recessive metabolic disease results from deficiencies of functional active enzyme known collectively as Glycogen storage disorder type V (GSDV) or McArdle disease (4,13,14). Pharmaceutical research on PYGM has focused on targeting the enzyme via gene and replacement therapy as well as small molecule treatments in McArdle disease, cardiac dysfunction, and osteosarcoma (15-18).

References:

1. Browner, M.F. and R.J. Fletterick. (1992) Trends. Biochem. Sci. **17**:66.
2. Crerar, M.M. *et al.* (1995) J. Biol. Chem. **270**:13748.
3. Lukacs, C.M. *et al.* (2006) Proteins. **63**:1123.
4. Migocka-Patrzalek, M. and M. Elias. (2021) Cells. **10**:883.
5. Hernández, C. *et al.* (2014) Acta Diabetol. **51**:543.
6. Jakobsen, E. *et al.* (2017) Neurochem. Res. **42**:2490.
7. Llavero, F. and J.L. Zugaza. (2024) Biochem. Soc. Trans. **52**:1265.
8. Arrizabalaga, O. *et al.* (2012) J. Biol. Chem. **287**:11878.
9. Llavero, F. *et al.* (2016) Cell Signal. **28**:1713.
10. Tang, Z. *et al.* (2017) Nucleic Acids Res. **45**:W98.
11. Jin, Y. and Y. Yang. (2019) Biosci. Rep. **39**:BSR20191612.
12. Xu, C. *et al.* (2025) Front. Immunol. **16**:1639303.
13. Llavero, F. *et al.* (2019) Int. J. Mol. Sci. **20**:5919.
14. Echaniz-Laguna, A. *et al.* (2020) Ann. Neurol. **88**:274.
15. McNamara, E.L. *et al.* (2020) Hum. Mol. Genet. **29**:20.
16. Villarreal-Salazar, M. *et al.* (2021) Genes (Basel). **13**:74.
17. Luo, L. *et al.* (2023) Mol. Med. **29**:36.
18. Gan, J. *et al.* (2025) Circulation. **152**:1146.

Bio-Techne®
Global | bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL: 1.612.379.2956
USA | TEL: 800.343.7475 Canada | TEL: 855.668.8722 Europe | Middle East | Africa TEL: +44.0.1235.529449
China | info.cn@bio-techne.com TEL: 400.821.3475