Recombinant Human PGM1 His-tag

Catalog Number: 11681-P1

DESCRIPTION

Source	*E. coli*-derived human PGM1 protein Val2-Thr562, with an N-terminal Met and 6-His tag Accession # P36871.3
N-terminal Sequence Analysis	Met
Predicted Molecular Mass	62 kDa

SPECIFICATIONS

SDS-PAGE	58-64 kDa, under reducing conditions
Activity	Measured by its ability to convert α-D-glucose 1-phosphate to α-D-glucose 6-phosphate in a coupled reaction. The specific activity is >6500 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: 25 mM Tris, 1 mM $MgCl_2$, pH 8.0
- Recombinant Human PGM1 His-tag (rhPGM1) (Catalog # 11681-PI)
- Coupling Enzyme: Recombinant Human G6PD His-tag (rhG6PD) (Catalog # 10096-DH)
- L-Cysteine, 50 mM stock in deionized water
- β-Nicotinamide adenine dinucleotide phosphate ($NADP^+$), 50 mM stock in deionized water
- Substrate: α-D-Glucose 1-phosphate, 10 mM stock in deionized water
- Clear 96-well Plate
- Plate Reader with Absorbance Read Capability

Assay

1. Create a Master Mix containing 2 mM L-Cysteine, 2 mM $NADP^+$, 5 µg/mL rhG6PD, and 0.1 µg/mL rhPGM1 in Assay Buffer. Include a Control containing the same master mix components in Assay Buffer, but without rhPGM1.
2. Incubate the Master Mixes and Control at room temperature for 10 minutes.
3. Dilute Substrate to 1 mM in Assay Buffer.
4. Following incubation, load 50 µL of each master mix (including the Control) to the plate and start the reaction by adding 50 µL of 1 mM Substrate to all wells.
5. Read plate at 340 nm (absorbance) in kinetic mode for 5 minutes.
6. Calculate specific activity:

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

*Adjusted for Control

**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:

- rhPGM1: 0.005 µg
- rhG6PD: 0.250 µg
- Substrate: 0.5 mM
- L-Cysteine: 1 mM
- $NADP^+$: 1 mM

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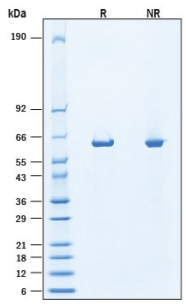
PREPARATION AND STORAGE

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

SDS-PAGE



Recombinant Human PGM1 His-tag Protein SDS-PAGE. 2 µg/lane of Recombinant Human PGM1 His-tag Protein (Catalog # 11681-P1) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 58-64 kDa, under reducing conditions.

BACKGROUND

Phosphoglucomutase-1 (PGM1) has a central role in metabolism as it mediates the switch between glycolysis and gluconeogenesis by catalyzing the interconversion of glucose 1-phosphate and glucose 6-phosphate within the nucleotide sugar, galactose, and pentose phosphate pathways and is involved in protein N-glycosylation (1). It is a member of the phosphohexose mutase family and is ubiquitously expressed in tissues and organs (1,2). PGM1 is a cytosolic, magnesium-dependent monomer with four domains that form an overall heart shape (1,2). The active site is located in a large central cleft in the middle of the four domains that contain a catalytic phosphoserine that participates in phosphoryl transfer, a metal binding loop, a sugar-binding loop, and a phosphate-binding site (2). Deficiency in PGM1 activity has been identified to cause glycogen storage disorder GSD XIV and is also a rare genetic disorder congenital disorder of glycosylation (CDG) called PGM1-CDG or CDG1T (3-6) with affected individuals showing multiple disease phenotypes including cardiomyopathy, muscular weakness, and hepatopathy (4,6,7). Some, but not all, clinical symptoms can be improved with oral D-galactose treatment (4,7,8). In addition, PGM1 is down-regulated in cancers such as hepatocellular carcinoma (HCC) and colorectal cancer (CRC) and up-regulated cancers such as lung and gastric cancer where it has been linked to lower survival in all cases making PGM1 a potential biomarker in several cancers (9-12). Galactose supplementation was not corrective in HCC suggesting PGM1's role in regulation of glycogen synthesis and glucose regulation is critical in promotion of tumor cell proliferation and growth (9,10,12,13). Given the key role PGM1 plays in metabolism in cancer and disease there is interest in PGM1 as a therapeutic target (10-12).

References:

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