Recombinant Human G6PD His-tag

Catalog Number: 10096-DH

DESCRIPTION

Source	*E. coli*-derived human G6PD protein Ala2-Leu515, with C-terminal 6x His tag Accession # P11413
Predicted Molecular Mass	60 kDa

SPECIFICATIONS

SDS-PAGE	56 kDa, reducing conditions
Activity	Measured by its ability to dehydrogenate glucose-6-phosphate. The specific activity is >14,000 pmol/min/µg, as measured under the described conditions.
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 Colloidal Coomassie® Blue stain.
Formulation	Supplied as a 0.2 µm filtered solution in Tris, NADP and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 25 mM Tris, 50 mM NaCl, 10 mM $MgCl_2$, pH 8.0.
- Recombinant Human G6PD His-tag (rhG6PD) (Catalog # 10096-DH).
- Donor Substrate: Glucose-6-phosphate sodium salt (Sigma, Catalog # G7879), 10 mM stock in deionized water.
- Acceptor Substrate: β-Nicotinamide adenine dinucleotide phosphate (NADP) (Sigma, Catalog # N5755), 50 mM stock in deionized water.
- 96-well Clear Plate (Catalog # DY990).
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent.

Assay

1. Dilute rhG6PD to 1 ng/µL in Assay Buffer.
2. Prepare substrate mixture containing 1 mM NADP and 1 mM Glucose-6-Phosphate in Assay Buffer.
3. Load in a plate 50 µL of 1 ng/µL rhG6PD, and start the reaction by adding 50 µL of substrate mixture. Include a Substrate Blank containing 50 µL of Assay Buffer and 50 µL of substrate mixture.
4. Read plate at 340 nm (absorbance) in kinetic mode for five minutes.
5. Calculate specific activity:

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

*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:

- rhG6PD: 0.05 µg
- NADP: 0.5 mM
- Glucose-6-phosphate: 0.5 mM

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.

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BACKGROUND

Glucose-6-phosphate dehydrogenase (G6PD) converts D-glucose 6-phosphate (G6P) into 6-phosphoglucono-δ-lactone and generate co-enzyme nicotinamide adenine dinucleotide phosphate (NADPH) (1). G6PD is the rate-limiting enzyme of the pentose phosphate pathway that supplies reducing energy to cells by maintaining the level of NADPH, which in turn maintains the level of glutathione in these cells that helps protect the red blood cells against oxidative damage from compounds like hydrogen peroxide (1, 2). More importantly, NADPH is used for biosynthesis of fatty acids or isoprenoids. G6PD is generally found as a dimer of two identical monomers (3). Depending on conditions, such as pH, these dimers can themselves dimerize to form tetramers. Each monomer in the complex has a substrate binding site that binds to G6P, and a catalytic coenzyme binding site that binds to $NADP^+$/NADPH using the Rossman fold (4). Its activity is stimulated by the substrate G6P and $NADP^+$. Clinically, genetic deficiency of G6PD predisposes a person to non-immune hemolytic anemia (5). G6PD is remarkable for its genetic diversity. Many variants of G6PD have been described with wide-ranging levels of enzyme activity and associated clinical symptoms. G6PD is frequently used as a coupling enzyme for measuring the enzymatic activity of glucose kinase (6).

References:

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