APA716Hu01 5mg Active Glucose-6-phosphate Dehydrogenase (G6PD) Organism Species: *Homo sapiens* (Human) *Instruction manual*

FOR RESEARCH USE ONLY NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

[PROPERTIES]

Source: Prokaryotic expression. Host: *E. coli* Residues: Met1~Leu515 Tags: N-terminal His-tag Purity: >90% Endotoxin Level: <1.0EU per 1µg (determined by the LAL method). Buffer Formulation: PBS, pH7.4, containing 0.01% SKL, 5%Trehalose . Original Concentration: 200µg/mL Applications: Cell culture; Activity Assays. (May be suitable for use in other assays to be determined by the end user.) Predicted isoelectric point: 6.5 Predicted Molecular Mass: 60.5kDa Accurate Molecular Mass: 60kDa as determined by SDS-PAGE reducing conditions.

[<u>USAGE</u>]

Reconstitute in 10mM PBS (pH7.4) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

[STORAGE AND STABILITY]

Storage: Avoid repeated freeze/thaw cycles.

Store at 2-8°C for one month.

Aliquot and store at -80°C for 12 months.

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Stability Test: The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

[SEQUENCE]

MAEQVALSRT QVCGILREEL FQGDAFHQSD THIFIIMGAS GDLAKKKIYP TIWWLFRDGL LPENTFIVGY ARSRLTVADI RKQSEPFFKA TPEEKLKLED FFARNSYVAG QYDDAASYQR LNSHMNALHL GSQANRLFYL ALPPTVYEAV TKNIHESCMS QIGWNRIIVE KPFGRDLQSS DRLSNHISSL FREDQIYRID HYLGKEMVQN LMVLRFANRI FGPIWNRDNI ACVILTFKEP FGTEGRGGYF DEFGIIRDVM QNHLLQMLCL VAMEKPASTN SDDVRDEKVK VLKCISEVQA NNVVLGQYVG NPDGEGEATK GYLDDPTVPR GSTTATFAAV VLYVENERWD GVPFILRCGK ALNERKAEVR LQFHDVAGDI FHQQCKRNEL VIRVQPNEAV YTKMMTKKPG MFFNPEESEL DLTYGNRYKN VKLPDAYERL ILDVFCGSQM HFVRSDELRE AWRIFTPLLH QIELEKPKPI PYIYGSRGPT EADELMKRVG FQYEGTYKWV NPHKL

[ACTIVITY]

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). 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. More importantly, NADPH is used for biosynthesis of fatty acids or isoprenoids. G6PD is generally found as a dimer of two identical monomers. 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+. The activity of recombinant human G6PD was measured by its

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ability to dehydrogenate glucose-6-phosphate. The reaction was performed in 25 mM Tris, 50 mM NaCl, 10 mM MgCl2, pH 8.0 (Assay Buffer), initiated by addition 50 μ L of various concentrations of G6PD (diluted by Assay Buffer) to 50 μ l substrate mixture consists of 1 mM NADP and 1 mM G6P. The final well serves as a negative control with no G6PD, replaced with 50 μ L assay buffer. Then read at a wavelength of 340 nm in kinetic mode for 5 minutes. The specific activity of recombinant human G6PD is >280 pmol/min/µg.

Specific Activity (pmol/min/ug)=

Adjusted Vmax* (OD/min) x well volume (L) x 1012 pmol/mol

ext. coeff** (M-1cm-1) x path corr.*** (cm) x amount of enzyme (ug)

*Adjusted for Substrate Blank **Using the extinction coefficient 6220 M⁻¹cm⁻¹ ***Using the path correction 0.320 cm

[IDENTIFICATION]



Figure 1. Gene Sequencing (extract)

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kDa 70
44
33
26
22
18
14
10

Figure 2. SDS-PAGE

Sample: Active recombinant G6PD, Human

[IMPORTANT NOTE]

The kit is designed for research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.