Technical Manual

ChromaDazzle Glucose-6-Phosphate (G6P) Assay Kit

Catalogue Code: BA0111

Pack Size: 100 assays

Research Use Only

DESCRIPTION

GLUCOSE-6-PHOSPHATE (G6P) is glucose sugar phosphorylated on carbon 6. Most of the glucose entering cells is phosphorylated to G6P. G6P has three primary fates within the cell. It lies at the start of two major metabolic pathways: glycolysis and the pentose phosphate pathway. In addition to these metabolic pathways, glucose 6-phosphate may also be converted to glycogen or starch for storage. The Assay Genie ChromaDazzle Glucose-6-Phosphate (G6P) Assay Kit provides a simple, and automation-ready procedure for measuring G6P concentration. G6P is oxidized by glucose-6-phosphate dehydrogenase and the formed NADPH is coupled to the formazan (WST-8) chromogen. The intensity of the product color, measured at 460 nm, is proportional to the G6P concentration in the sample.

KEY FEATURES

Fast and sensitive. Linear detection range: 10 to 1000 µM G6P.

Convenient and high-throughput. Homogeneous "mix-incubate-measure" type assay. Can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

APPLICATIONS

G6P determination in biological samples (e.g. plasma, serum, tissue and culture media.)

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer: 10 mL **Enzyme A:** 120 µL
NADP/WST8: 1 mL **Enzyme B:** 120 µL
Standard (100 mM G6P): 100 µL

Storage conditions. The kit is shipped on ice. Store all kit components at -20 °C. Shelf life of six months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Sample Preparation

Tissue or cell samples (2 x10^6) can be homogenized in 100 µL PBS. Centrifuge at 14,000 rpm for 5 min. Use clear supernatant for assay.

Serum and plasma samples can be measured directly but may need a sample blank if they have significant absorbance at 460 nm.

Assay Procedure

1. *Standards*. Dilute the G6P Standard to 1000 µM Premix by mixing 5 µL of the 100 mM Standard with 495 µL dH_2O. Next, dilute standards in 1.5-mL centrifuge tubes as described in the table.

No	Premix + dH_2O	G6P (µM)
1	100 µL + 0 µL	1000
2	60 µL + 40 µL	600
3	30 µL + 70 µL	300
4	0 µL + 100 µL	0

Transfer 20 µL of each standard to separate wells in a 96 well plate.

2. *Samples.* Add 20 µL of each sample to separate wells in a 96 well plate. (For samples that may have background absorbance at 460 nm or significant levels of NADH or NADPH (> 20 µM), add 20 µL of the sample to a second well to serve as a sample blank).
3. *G6P Detection*. Prepare enough working reagent (WR) for all standards and samples. For each reaction combine the following: 75 µL Assay Buffer, 8 µL NADP/WST8, 1 µL Enzyme A and 1 µL Enzyme B. If including Sample Blanks, prepare a

blank working reagent (BWR) ***without*** the Enzyme A. Add 80 µL of the appropriate WR to each Standard and Sample well. Mix well and incubate protected from light for 20 min at RT.

4. Read OD_{460nm}.

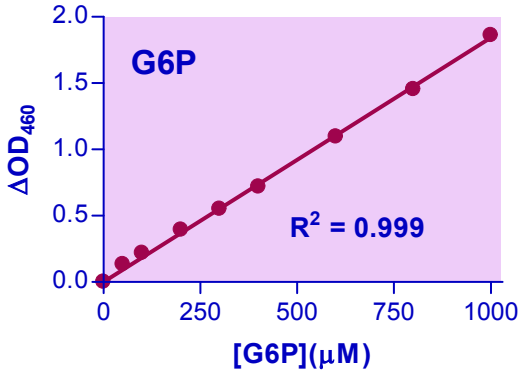
CALCULATION

Subtract the blank value (#4) from the standard values and plot the ΔOD against standard concentrations. Determine the slope and calculate the G6P concentration of the Samples as follows:

$$[G6P] = \frac{OD_{SAMPLE} - OD_{BLANK}}{Slope} \times n \quad (\mu M)$$

where OD_{SAMPLE} is the OD reading of the Sample and OD_{BLANK} is the OD reading of the Blank (Standard #4) or Sample Blank if used. *n* is the sample dilution factor. If the calculated G6P concentration is >1000 µM, dilute sample in dH_2O and repeat assay. Multiply result by the dilution factor.

Conversions: 100 µM G6P equals 34 mg/L, 0.0034% or 34 ppm.



Standard Curve in 96-well plate assay

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices and accessories (e.g. multi-channel pipettor), clear flat-bottom 96-well plates (e.g. VWR cat# 82050-760), centrifuge tubes and plate reader.

LITERATURE

1. Zhu, A et al (2011). An enzymatic colorimetric assay for glucose-6-phsphate. Anal. Biohem. 419:266-70.
2. zhu, a et al (2009). an enzymatic fluorimetric assay for glucose-6-phsphate application in an in vitro warburg-like effect. anal. biohem. 388:97-101.
3. Cabell, la et al (1999). a competition assay for determining glucose-6-phosphate concentration with a *tris*-boronic acid receptor. tetrahedron lett.40: 7753-6.

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