# EnzyChrom™ Phosphoglucomutase Assay Kit (EPGM-100)

## Quantitative Colorimetric Assay for Phosphoglucomutase Activity

## DESCRIPTION

*PHOSPHOGLUCOMUTASE* (E.C. 5.4.2.2) is an enzyme which catalyzes the interconversion of glucose-1-phosphate (G1P) and glucose-6-phosate (G6P). Phosphoglucomutase (PGM) plays a crucial role in glycogen synthesis and degradation. PGM 1 deficiency can cause a rare congenital disorder of glycosylation. A decrease in PGM activity has also been linked with oxidative stress. In BioAssay Systems' EPGM-100 assay, PGM converts G1P to G6P, which is then oxidized by glucose-6-phosphate dehydrogenase. The formed NADPH is coupled to a formazan chromogen. The increase in absorbance at 460 nm is directly proportional to phosphoglucomutase activity.

## KEY FEATURES

**Safe and sensitive.** Non-radioactive assay. Linear detection range in 96-well plate: 0.5 to 20 U/L activity.

**Fast and convenient**. The procedure involves addition of a single working reagent and incubation for 20 min. Room temperature assay. No 37°C incubator is needed.

**High-throughput**. Homogeneous "mix-incubate-measure" type assay. Can be readily automated to assay thousands of samples per day.

## APPLICATIONS

For quantitative determination of phosphoglucomutase enzyme activity in biological samples.

## 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 | **Substrate:** | 800 µL |

**Storage conditions**. The kit is shipped on ice. Store all components at -20°C upon receiving. Shelf life: 6 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

This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Working Reagent to samples should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended. Assays can be performed at any desired temperature (e.g. 25°C or 37°C).

**Sample Preparation:** Serum and plasma can be assayed directly.

*Cell/tissue Lysate*: Homogenize sample in an appropriate volume of cold PBS. Centrifuge at 10,000 x g for 15 min at 4°C and use supernatant for assay.

**Standard Preparation:** Prepare a 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 + $dH_2O$ | Total Volume (µL) | G6P (µM) |
|---|---|---|---|
| 1 | 100 µL + 0 µL | 100 µL | 1000 |
| 2 | 60 µL + 40 µL | 100 µL | 600 |
| 3 | 30 µL + 70 µL | 100 µL | 300 |
| 4 | 0 µL + 100 µL | 100 µL | 0 |

*Standards:* Transfer 20 µL of each Standard dilution to separate wells of a clear 96-well plate.

**Reagent Preparation:** Keep thawed enzymes on ice and equilibrate all other reagents to desired reaction temperature (e.g. 25°C or 37°C). Briefly centrifuge tubes before use.

Prepare enough Working Reagent (WR) for all standards and samples by mixing, for each well, 70 µL Assay Buffer, 8 µL NADP/WST8, 8 µL Substrate, 1 µL Enzyme A, and 1 µL Enzyme B. Fresh reconstitution of the WR is recommended.

**Reaction Preparation:**

1. Transfer 20 µL of each sample to separate wells.
2. Add 80 µL WR to all assay wells. Tap plate briefly to mix.
3. Incubate at desired reaction temperature. Use a plate reader to read $OD_{460nm}$ at 5 minutes ($OD_5$), and again at 20 minutes ($OD_{20}$). Alternatively, immediately after adding WR and mixing, record kinetics at $OD_{460nm}$ at desired reaction temperature for at least 20 min.

## CALCULATION

Using the OD values from 20 min, subtract the blank value (Standard #4) from the standard values and plot ΔOD against the standard concentrations. Determine the slope ($\mu M^{-1}$) of the G6P standard curve and calculate the PGM activity in each sample as follows:

$$\text{PGM Activity} = \frac{OD_{20} - OD_5}{\text{Slope } (\mu M^{-1}) \times t\,(\text{min})} \times n \ \text{(U/L)}$$

where $OD_{20}$ and $OD_5$ are the $OD_{460nm}$ values at 20 min and 5 min, respectively. *t* is the reaction time (15 min). *n* is the dilution factor.

*Unit definition*: 1 Unit (U) of phosphoglucomutase will catalyze the conversion of 1 µmole of glucose-1-phosphate to glucose-6-phosate per min at pH 8.2.

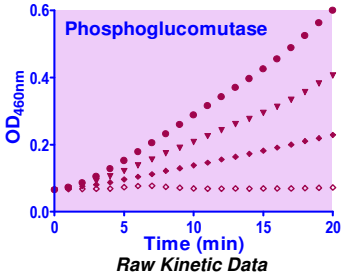
*Note: If sample PGM activity exceeds 20 U/L, either use a shorter reaction time or dilute samples in water and repeat the assay. If kinetics are recorded, any two time points in which the activity remains linear can be chosen for analysis. Use OD values from the latter time point to calculate the slope from the standards and adjust* t *to the chosen time interval.*

## MATERIALS REQUIRED, BUT NOT PROVIDED

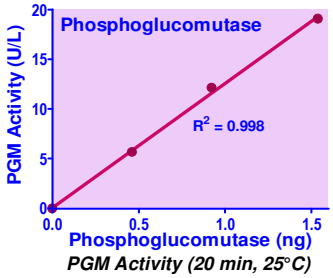
Pipetting devices and accessories (e.g. multi-channel pipettor), clear flat-bottom 96-well plates (e.g. VWR cat# 82050-760), centrifuge tubes and plate reader.

## EXAMPLES

PGM activity was determined in at least duplicate following the protocol. The values were 11.2 ± 0.4 U/L for bovine serum, 1.8 ± 0.03 U/L for canine plasma, 23.6 ± 1.8 U/L for PANC-1 cell lysate. These values are examples and are not intended as expected values.



*Raw Kinetic Data*



*PGM Activity (20 min, 25°C)*

## LITERATURE

1. Altassan, R et al (2021). International consensus guidelines for phosphoglucomutase 1 deficiency (PGM1-CDG): Diagnosis, follow-up, and management. Journal of inherited metabolic disease. 44(1):148-63.
2. Ashida, H et al (1991). Relationship between Hepatic Phosphoglucomutase Activity and Oxidative Stress Caused by Dietary Products of Lipid Peroxidation. Agricultural and Biological Chemistry. 55(7):1765–70.
3. Zhu, A et al (2011). An enzymatic colorimetric assay for glucose-6-phsphate. Anal. Biohem. 419:266-70.