

# Glucose-6-Phosphate Dehydrogenase (G6PDH) Assay Kit (Colorimetric)

LS-K280-100 (100 Tests) • Store at -20°C



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## Introduction

GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PDH) is a cytosolic enzyme in the pentose phosphate pathway which supplies reducing energy to cells by maintaining the level of the co-enzyme nicotinamide adenine dinucleotide phosphate (NADPH). G6PDH reduces nicotinamide adenine dinucleotide phosphate (NADP) to NADPH while oxidizing glucose-6-phosphate (G6P). Humans with a genetic deficiency of G6PDH are predisposed to non-immune hemolytic anemia. This non-radioactive, colorimetric G6PDH assay is based on the reduction of the tetrazolium salt MTT in a NADPH-coupled enzymatic reaction to a reduced form of MTT which exhibits an absorption maximum at 565 nm. The increase in absorbance at 565 nm is proportional to the enzyme activity.

## Key Features

- Fast and sensitive. Linear detection range (20 µL sample): 0.2 to 100 U/L for 15 min reaction.
- 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

- G6PDH activity determination in biological samples (e.g. plasma, serum, erythrocytes, tissue and culture media.)

## Components

Component	K280-100
	100 Tests
Assay Buffer	10 mL
NADP/MTT	1 mL
Substrate	1 mL
Diaphorase	120 µL
Calibrator	1.5 mL

## Materials Not Supplied

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.

## Storage

The kit is shipped at ambient temperature. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

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**FOR RESEARCH USE ONLY! Not for use in humans.**

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## Assay Procedure

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 executed at any desired temperature (e.g. 25°C or 37°C).

### Sample Preparation

Serum and plasma are assayed directly.

Tissue: prior to dissection, rinse tissue in phosphate buffered saline (pH 7.4) to remove blood. Homogenize tissue (50 mg) in ~200 µL buffer containing 50 mM potassium phosphate (pH 7.5). Centrifuge at 10,000 × g for 15 min at 4°C. Remove supernatant for assay.

Cell Lysate: collect cells by centrifugation at 2,000 × g for 5 min at 4°C. For adherent cells, do not harvest cells using proteolytic enzymes; rather use a rubber policeman. Homogenize or sonicate cells in an appropriate volume of cold buffer containing 50 mM potassium phosphate (pH 7.5). Centrifuge at 10,000 × g for 15 min at 4°C. Remove supernatant for assay.

All samples can be stored at -20 to -80°C for at least one month.

### Reagent Preparation

Equilibrate reagents to desired reaction temperature (e.g. 25°C or 37°C). Briefly centrifuge tubes before use.

Prepare enough Working Reagent (WR) for all assay wells by mixing, for each 96-well assay: 8 µL Substrate, 8 µL NADP/MTT Solution, 1 µL Diaphorase and 70 µL Assay Buffer.

### Procedure

1. Transfer 100 µL H<sub>2</sub>O (OD<sub>H2O</sub>) and 100 µL Calibrator (OD<sub>CAL</sub>) solution into wells of a clear flat-bottom 96-well plate.
2. Transfer 20 µL of each sample into separate wells and then add 80 µL WR to each sample well. Tap plate briefly to mix.
3. Read OD<sub>565nm</sub> (OD<sub>0</sub>), and again after 15 min (OD<sub>15</sub>) on a plate reader.

### Calculations

Subtract the OD<sub>0</sub> from OD<sub>15</sub> for each sample to compute the ΔOD<sub>s</sub> values. G6PDH activity can then be calculated as follows:

$$\begin{aligned} \text{G6PDH Activity} &= \frac{\Delta \text{OD}_s}{\epsilon_{\text{mtt}} \cdot l} \times \frac{\text{Reaction Vol } (\mu\text{L})}{t \text{ (min)} \cdot \text{Sample Vol } (\mu\text{L})} \times n \\ &= \frac{\Delta \text{OD}_s}{\text{OD}_{\text{CAL}} - \text{OD}_{\text{H}_2\text{O}}} \times \frac{273}{t \text{ (min)}} \times n \quad (\text{U/L}) \end{aligned}$$

where  $\epsilon_{\text{mtt}}$  is the molar absorption coefficient of reduced MTT.  $l$  is the light path length which is calculated from the calibrator. OD<sub>CAL</sub> and OD<sub>H2O</sub> are OD<sub>565nm</sub> (OD<sub>0</sub>) values of the Calibrator and water.  $t$  is the reaction time (15 min is the recommended time). Reaction Vol and Sample Vol are 100 µL and 20 µL, respectively.  $n$  is the dilution factor.

Unit definition: 1 Unit (U) of G6PDH will catalyze the conversion of 1 µmole of NADP to NADPH per min at pH 8.2.

Note: If sample G6PDH activity exceeds 100 U/L, either use a shorter reaction time or dilute samples in water and repeat the assay. For samples with G6PDH activity < 1 U/L, the incubation time can be extended to 2 hours.

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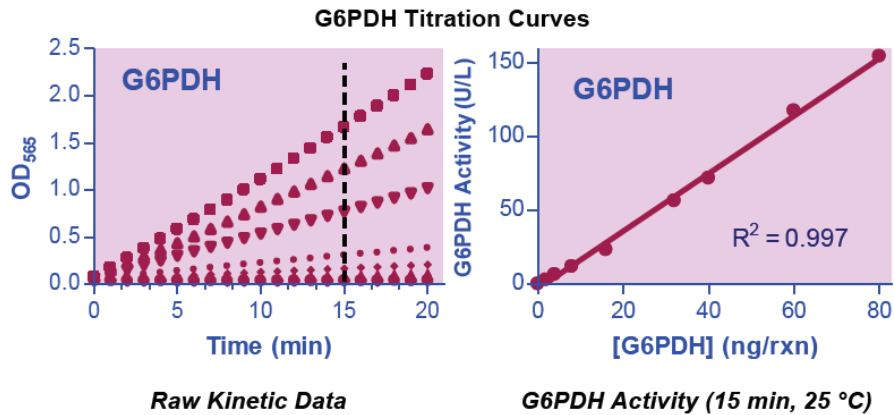
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## Sample Data



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