# Glucose-6-Phosphate Dehydrogenase Assay Kit

Catalog Number KA0880

100 assays

Version: 03

Intended for research use only

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## Introduction

### Background

Glucose-6-phosphate dehydrogenase (G6PDH) is a cytosolic enzyme in the pentose phosphate pathway, a metabolic pathway that supplies reducing energy to cells (such as erythrocytes) by maintaining the level of the co-enzyme nicotinamide adenine dinucleotide phosphate (NADPH). The NADPH in turn maintains the level of glutathione in these cells that helps protect the red blood cells against oxidative damage. Of greater quantitative importance is the production of NADPH for tissues actively engaged in biosynthesis of fatty acids and/or isoprenoids, such as the liver, mammary glands, adipose tissue, and the adrenal glands. The Glucose-6-Phosphate Dehydrogenase Assay Kit is a simple, sensitive and rapid assay detects the activity of G6PDH in a variety of samples. In the assay, glucose-6-phosphate is oxidized with the generation of a product which is utilized to convert a nearly colorless probe to an intensely colored product with an absorbance at 450nm. The G6PDH Assay Kit can detect as low as 0.04mU G6PDH per well.

## General Information

### Materials Supplied

List of component

| Component | Amount |
|---|---|
| G6PDH Assay Buffer | 25 ml |
| G6PDH Substrate: lyophilized. | 1 vial |
| G6PDH Developer: lyophilized. | 1 vial |
| G6PDH Positive Control: lyophilized. | 1 vial |
| NADH Standard (0.5 µmol): lyophilized | 1 vial |

### Storage Instruction

Store kit at -20°C, protect from light. Warm Assay Buffer to room temperature before use. Briefly centrifuge all small vials prior to opening.

## Assay Protocol

### Reagent Preparation

- ✓ G6PDH Substrate Mix: Reconstitute with 0.22 ml of Assay Buffer and mix thoroughly. Stable for 2 months at 4°C.
- ✓ G6PDH Developer: Dissolve with 0.22 ml $dH_2O$. Pipette up and down to dissolve. Stable for 2 months at -20°C.
- ✓ G6PDH Positive Control: Dissolve in 100 µl G6PDH Assay Buffer and mix thoroughly. Aliquot some amount into each vial, avoid freeze/thaw cycles. Keep cold while in use. Stable for 2 months at -20°C.
- ✓ NADH Standard: Dissolve in 400 µl $dH_2O$ to generate 1.25 mM (1.25 nmol/µl) NADH Standard solution. Keep cold while in use. Store at -20°C.

### Assay Procedure

1. Tissue or erythrocyte Sample Preparation:
   Samples (10-100 mg) should be rapidly homogenized with an equivalent volume of ice cold PBS or other buffer (pH 6.5-8). Add 1-50 µl samples into duplicate wells of a 96-well plate and bring volume to 50 µl with Assay Buffer. We suggest testing several doses of your samples to ensure readings are within the linear range.
2. Dilute Positive Control: Take 10 µl of the Positive Control and add 990 µl Assay Buffer. This should be a suitable dilution to get 0.1-1.0 OD in 30 minutes of incubation. Use 1-10 µl of the diluted Positive Control; adjust final volume to 50 µl with Assay Buffer.
3. Develop: Mix enough reagent for the number of samples and standards to be performed: For each well, prepare a total 50 µl Reaction Mix containing:

   | | Reaction Mix |
   |---|---|
   | G6PDH Assay Buffer | 46 µl |
   | G6PDH Substrate | 2 µl |
   | G6PDH Developer | 2 µl |

   Add 50 µl of the Reaction Mix to each well containing the Positive Control or test samples. Measure O.D. 450 nm at $T_1$ to read $A_1$, measure O.D. 450 nm again at $T_2$ after incubating the reaction at 37°C for 30 min (or longer if the G6PDH activity is low) to read $A_2$, protect from light. $\Delta A450\ nm = A_2 - A_1$.
   *Note: It is essential to read $A_1$ and $A_2$ in the reaction linear range. It will be more accurate if you read the reaction kinetics. Then choose $A_1$, $A_2$, in the reaction linear range.*
4. NADH Standard Curve: Add 0, 2, 4, 6, 8, and 10µl of the 1.25 mM NADH Standard into 96-well plate in duplicate to generate 0, 2.5, 5.0, 7.5, 10.0, and 12.5 nmol/well standard. Bring the final volume to 50 µl with Assay Buffer, and then add 50 µl Reaction Mix to each standard, mix well. Measure at O.D.450 nm.

## Data Analysis

### Calculation of Results

Subtract the background, plot NADH standard Curve. Apply the $\Delta A_{450nm}$ to the standard curve to get B (the NADH amount that was generated between $T_1$ and $T_2$).

$$\text{G6PDH Activity} = \frac{B}{(T_2\text{-}T_1) \times V} \times \text{Sample dilution} = \text{nmol/min/ml} = \text{mU/mL}$$

Where:

B is the NADH amount that was generated between $T_1$ and $T_2$ (in nmol).

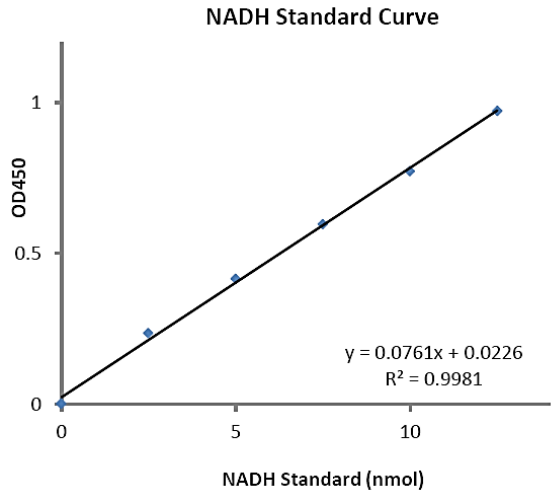
$T_1$ is the time of first reading ($A_1$) (in min).

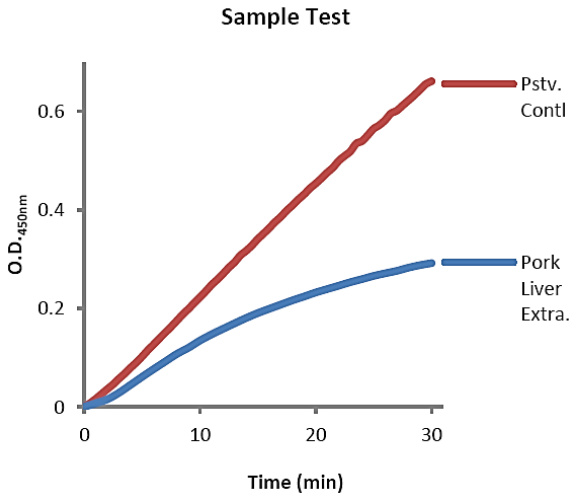
$T_2$ is the time of second reading ($A_2$) (in min).

V is the pretreated sample volume added into the reaction well (in ml).

Unit Definition

One unit defines as the amount of enzyme that catalyzes the conversion of 1.0µmol of glucose-6-phosphate into 6-phosphoglucono-δ-lactone and generates 1.0 µmol of $NAD^+$ to NADH per minute at 37°C.



## Resources

**Troubleshooting**

| Problems | Cause | Solution |
|---|---|---|
| Assay not working | • Use of ice-cold assay buffer.<br>• Omission of a step in the protocol.<br>• Plate read at incorrect wavelength.<br>• Use of a different 96-well plate. | • Assay buffer must be at room temperature.<br>• Refer and follow the data sheet precisely.<br>• Check the wavelength in the datasheet and the filter settings of the instrument.<br>• Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimeters: Clear plates. |
| Samples with erratic readings | • Use of an incompatible sample type.<br>• Sample prepared in a different buffer.<br>• Cell/tissue samples were not completely homogenized.<br>• Samples used after multiple free-thaw cycles.<br>• Presence of interfering substance in the sample.<br>• Use of old inappropriately stored samples. | • Refer to data sheet for details about incompatible samples.<br>• Use the assay buffer provided in the kit or refer data sheet for instructions.<br>• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope.<br>• Aliquot and freeze samples if needed to use multiple times.<br>• Troubleshoot if needed.<br>• Use fresh samples or store at correct temperature until use. |
| Lower/Higher readings in Samples and Standards | • Improperly thawed components.<br>• Use of expired kit or improperly stored reagents.<br>• Allowing the reagents to sit for extended times on ice.<br>• Incorrect incubation times or temperatures.<br>• Incorrect volumes used. | • Thaw al components completely and mix gently before use.<br>• Always check the expiry date and store the components appropriately.<br>• Always thaw and prepare fresh reaction mix before use<br>• Refer to datasheet and verify correct incubation times and temperatures.<br>• Use calibrate pipettes and aliquot correctly. |
| Reading do not follow a linear pattern for Standard curve | • Use of partially thawed components.<br>• Pipetting errors in the standard.<br>• Pipetting errors in the reaction mix.<br>• Air bubbles formed in well.<br>• Standard stock is at an incorrect concentration. | • Thaw and resuspend all components before preparing the reaction mix.<br>• Avoid pipetting small volumes.<br>• Prepare a master reaction mix whenever possible.<br>• Pipette gently against the wall of the tubes. |

| | | |
|---|---|---|
| | • Calculation errors.<br>• Substituting reagents from older kits/lots. | • Always refer the dilutions in the data sheet.<br>• Recheck calculations after referring the data sheet.<br>• Use fresh component from the same kit. |
| Unanticipated results | • Measured at incorrect wavelength.<br>• Samples contain interfering substances.<br>• Use of incompatible sample type.<br>• Sample reading above/below the linear range. | • Check the equipment and the filter setting.<br>• Troubleshoot if it interferes with the kit.<br>• Refer data sheet to check if sample is compatible with the kit or optimization is needed.<br>• Concentrate/Dilute sample so as to be in the linear range. |

*Note: The most probable list of causes is under each problem section. Causes/Solutions may overlap with other problems.*