Glucose-6-Phosphate Dehydrogenase Activity Colorimetric Assay Kit

(Catalog #K757-100; 100 assays; Store Kit at -20°C)

I. Introduction:

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. BioVision's 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 450 nm. The G6PDH Assay Kit can detect as low as 0.04 mU G6PDH per well.

II. Kit Contents:

Components	K757-100	Cap Code	Part Number
G6PDH Assay Buffer	25 ml	WM	K757-100-1
G6PDH Substrate (lyophilized)	1 vial	Orange	K757-100-2
G6PDH Developer (lyophilized)	1 vial	Red	K757-100-3
G6PDH Positive Control (lyophilized)	1 vial	Green	K757-100-4
NADH Standard (0.5 µl mol; lyophilized)	1 vial	Yellow	K757-100-5

III. Storage and Handling:

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

IV. Reagent Preparation and Storage Conditions:

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.

V. Assay Protocol:

1. **Tissue or erythrocyte Sample Preparation:** Samples (10 - 100mg) should be rapidly homogenized with an equivalent volume of ice cold PBS or other buffer (pH 6.5 - 8). Add 1 - 50µl 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 OD 450 nm at T_1 to read A_1, measure OD 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. $\Delta A450\ nm = A_2 - A_1$.

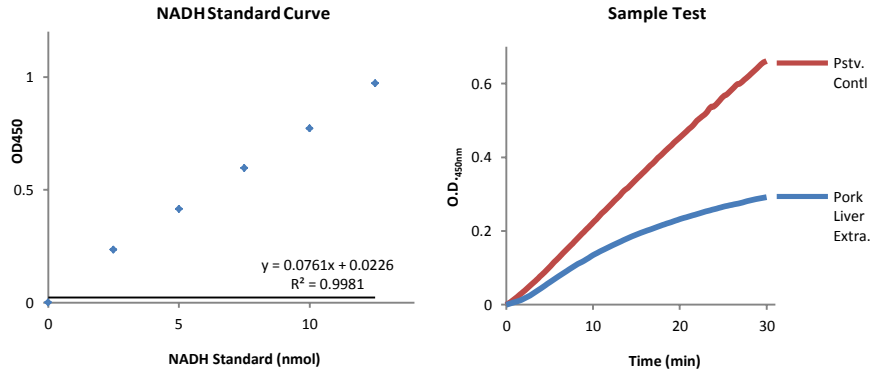
Note: It is essential to read A_1 and A_2 in the reaction linear range. It will be more accurate if you read the reaction kinetics. Then choose A_1, A_2, in the reaction linear range.

4. **NADH Standard Curve:**
Add 0, 2, 4, 6, 8, and 10 µl 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 50µl with Assay Buffer, and then add 50 µl Reaction Mix to each standard, mix well. Measure at OD 450 nm.
5. **Calculation:** Subtract the background, plot NADH standard Curve. Apply the ΔA_{450nm} to the standard curve to get B (the NADH amount that was generated between T_1 and T_2).

$$\text{G6PDH Activity} = \frac{B}{(T2-T1)\times V} \times \text{Sample dilution} = \text{nmol/min/ml} = \text{mU/mL}$$

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 1.0 µlmol of glucose-6-phosphate into 6-phosphoglucono-δ-lactone and generates 1.0 µmol of NAD^+ to NADH per minute at 37°C.



RELATED PRODUCTS:

Apoptosis Detection Kits & Reagents	Cell Proliferation & Senescence Kits
Glucose and Sucrose Assay Kit	Cholesterol, LDL/HDL Assay Kits
Glutathione Assay Kit	Ethanol and Uric Acid Assay Kit
NAD/NADH and NADP/NADPH Assay Kit	Lactate Assay Kits
TAC Total Antioxidant Capacity	Mono or Polysaccharide Assay Kits
Malic acid Assay Kit	Pyruvate Assay Kit

FOR RESEARCH USE ONLY! Not to be used on humans.

GENERAL TROUBLESHOOTING GUIDE:

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