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Product Information

Glucose-6-Phosphate Dehydrogenase Assay Kit

Catalog Number **MAK015**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Glucose-6-phosphate dehydrogenase (G6PDH) catalyzes the conversion of glucose-6-phosphate to 6-phosphoglucono-δ-lactone, the first and rate-limiting step of the pentose phosphate pathway (PPP). The PPP pathway is critical for maintaining the cofactor nicotinamide adenine dinucleotide phosphate (NADPH) and for the production of pentose sugars. The NADPH produced is critical for redox regulation via the regeneration of GSH and for providing reducing equivalents for fatty acid biosynthesis. Deficiencies in G6PDH predisposes individuals to non-immune hemolytic anemia.

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 this kit, glucose-6-phosphate is oxidized to generate a product, which is specifically detected by colorimetric (450 nm) assay. The G6PDH Assay Kit can detect as low as 0.04 milliunit of G6PDH per well.

Components

The kit is sufficient for 100 assays in 96 well plates.

G6PDH Assay Buffer Catalog Number MAK015A	25 mL
G6PDH Substrate Catalog Number MAK015B	1 vl
G6PDH Developer Catalog Number MAK015C	1 vl
G6PDH Positive Control Catalog Number MAK015D	1 vl
NADH Standard, 0.5 µmole Catalog Number MAK015E	1 vl

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

G6PDH Assay Buffer – Allow buffer to come to room temperature before use.

G6PDH Substrate Mix – Reconstitute in 220 µL of G6PDH Assay Buffer. Mix well by pipetting. Once reconstituted, the Substrate Mix is stable for two months at 4 °C.

G6PDH Developer – Reconstitute in 220 µL of water. Mix well by pipetting, and then aliquot and store at –20 °C. Use within 2 months of reconstitution.

G6PDH Positive Control – Reconstitute in 100 µL of G6PDH Assay Buffer. Mix well by pipetting and keep cold while in use. Aliquot and store at –20 °C. Use within 2 months of reconstitution.

NADH Standard – Reconstitute in 400 µL of water to generate a 1.25 mM standard solution. Mix well by pipetting and keep cold while in use. Aliquot and store at –20 °C. Use within 2 months of reconstitution.

Storage/Stability

The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

NADH Standards for Colorimetric Detection
Add 0, 2, 4, 6, 8, and 10 µL of the 1.25 mM NADH standard solution in duplicate into a 96 well plate, generating 0 (blank), 2.5, 5, 7.5, 10, and 12.5 nmole/well standards. Add Assay Buffer to a final volume of 50 µL.

Sample Preparation
Tissue (10–20 mg), cells (1×10^6), or erythrocyte samples (10–100 mg) should be rapidly homogenized in equivalent volumes of ice cold PBS or other buffer (pH 6.5–8). Centrifuge at 15,000 × *g* for 10 minutes to remove insoluble materials. Add 1–50 µL samples into duplicate wells of a 96-well plate. Bring samples to a final volume of 50 µL with G6PDH Assay Buffer.

For unknown samples, it is suggested to test several sample volumes to make sure the readings are within the standard curve range.

Dilute 10 µL of the Positive Control with 990 µL of Assay Buffer. Add 1–10 µL of the diluted Positive Control into duplicate wells of 96 well plate. Bring to final volume of 50 µL with G6PDH Assay Buffer. This should be a suitable dilution to get 0.1–1.0 A_{450} in 30 minutes of incubation.

Assay Reaction
1. Set up the Master Reaction Mix according to the scheme in Table 1. 50 µL of the Master Reaction Mix is required for each reaction (well).

Table 1.
Master Reaction Mix

Reagent	Master Reaction Mix
G6PDH Assay Buffer	46 µL
G6PDH Substrate Mix	2 µL
G6PDH Developer Mix	2 µL

2. Add 50 µL of the Master Reaction Mix to each of the standard, positive control, and sample wells. Protect the plate from light. Mix well using a horizontal shaker or by pipetting.

3. After 2–3 minutes, take the initial measurement ($T_{initial}$). Measure the absorbance at 450 nm at the initial time $(A_{450})_{initial}$.
   Note: It is essential $(A_{450})_{initial}$ is in the linear range of the standard curve.

4. Incubate the plate at 37 °C taking measurements (A_{450}) every 5 minutes. Protect the plate from light during the incubation.

5. Continue taking measurements until the value of the most active sample is greater than the value of the highest standard (12.5 nmole/well). At this time the most active sample is near or exceeds the end of the linear range of the standard curve.

6. The final measurement $[(A_{450})_{final}]$ for calculating the enzyme activity would be penultimate reading or the value before the most active sample is near or exceeds the end of the linear range of the standard curve, see step 5. The time of the penultimate reading is T_{final}.
   Note: It is essential the final measurement falls within the linear range of the standard curve.

Results

Calculations

Correct for the background by subtracting the final measurement [$(A_{450})_{final}$] obtained for the 0 (blank) NADH standard from the final measurement [$(A_{450})_{final}$] of the standards. Background values can be significant and must be subtracted from all readings. Plot the NADH standard curve.

Note: A new standard curve must be set up each time the assay is run.

Calculate the change in measurement from $T_{initial}$ to T_{final} for the samples.

$$\Delta A_{450} = (A_{450})_{final} - (A_{450})_{initial}$$

Compare the ΔA_{450} of each sample to the standard curve to determine the amount of NADH generated by the kinase assay between $T_{initial}$ and T_{final} (B).

The G6PDH activity of a sample may be determined by the following equation:

$$\text{G6PDH Activity} = \frac{B \times \text{Sample Dilution Factor}}{(\text{Reaction Time}) \times V}$$

B = Amount (nmole) of NADH generated between $T_{initial}$ and T_{final}.

Reaction Time = $T_{final} - T_{initial}$ (minutes)

V = sample volume (mL) added to well

G6PDH activity reported as nmole/min/mL (milliunit/mL)

One unit is the amount of enzyme that catalyzes the conversion of 1.0 μmole of glucose-6-phosphate to 6-phosphoglucono-δ-lactone and generates 1.0 μmole of NADH per minute at 37 °C.

Example:

NADH amount (B) = 5.84 nmole

First reading ($T_{initial}$) = 3 minute

Second reading (T_{final}) = 32 minutes

Sample volume (V) is 0.01 mL

Sample dilution is 1

G6PDH activity is:

$$\frac{5.84}{(32 - 3) \times 0.01} \times 1 = 20.14 \text{ milliunits/mL}$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay Not Working	Assay Buffer Ice Cold	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For fluorescence assays, use black plates with clear bottoms. For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/Higher Readings in Samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Always check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Always prepare fresh Master Reaction Mix before use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear Standard Curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the reaction mix	Prepare a Master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to the dilutions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
		Deproteinize samples
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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