**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 predispose 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**
  - 25 mL
  - Catalog Number MAK015A

- **G6PDH Substrate**
  - 1 vl
  - Catalog Number MAK015B

- **G6PDH Developer**
  - 1 vl
  - Catalog Number MAK015C

- **G6PDH Positive Control**
  - 1 vl
  - Catalog Number MAK015D

- **NADH Standard, 0.5 μmole**
  - 1 vl
  - Catalog Number MAK015E

**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 \((1 \times 10^6)\), 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 \(\times g\) 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

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Master Reaction Mix</th>
</tr>
</thead>
<tbody>
<tr>
<td>G6PDH Assay Buffer</td>
<td>46 μL</td>
</tr>
<tr>
<td>G6PDH Substrate Mix</td>
<td>2 μL</td>
</tr>
<tr>
<td>G6PDH Developer Mix</td>
<td>2 μL</td>
</tr>
</tbody>
</table>

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 \(T_{\text{initial}}\). Measure the absorbance at 450 nm at the initial time \((A_{450})_{\text{initial}}\).

Note: It is essential \((A_{450})_{\text{initial}}\) 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 \([A_{450}]_{\text{final}}\) 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 \(T_{\text{final}}\).

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 \([A_{450}]_{\text{final}}\) obtained for the 0 (blank) NADH standard from the final measurement \([A_{450}]_{\text{final}}\) 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 \(T_{\text{initial}}\) to \(T_{\text{final}}\) for the samples.

\[\Delta A_{450} = (A_{450})_{\text{final}} - (A_{450})_{\text{initial}}\]

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

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

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

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

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

\(V = \text{sample volume (mL) added to well}\)

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 \((T_{\text{initial}}) = 3 \text{ minute}\)
Second reading \((T_{\text{final}}) = 32 \text{ minutes}\)
Sample volume \((V)\) is 0.01 mL
Sample dilution is 1

G6PDH activity is:

\[
\frac{5.84 \times 1}{(32 - 3) \times 0.01} = 20.14 \text{ milliunits/mL}
\]
<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Cause</th>
<th>Suggested Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay Not Working</td>
<td>Assay Buffer Ice Cold</td>
<td>Assay Buffer must be at room temperature</td>
</tr>
<tr>
<td></td>
<td>Omission of step in procedure</td>
<td>Refer and follow Technical Bulletin precisely</td>
</tr>
<tr>
<td></td>
<td>Plate reader at incorrect wavelength</td>
<td>Check filter settings of instrument</td>
</tr>
<tr>
<td></td>
<td>Type of 96 well plate used</td>
<td>For fluorescence assays, use black plates with clear bottoms. For colorimetric assays, use clear plates</td>
</tr>
<tr>
<td>Samples with erratic readings</td>
<td>Samples prepared in different buffer</td>
<td>Use the Assay Buffer provided or refer to Technical Bulletin for instructions</td>
</tr>
<tr>
<td></td>
<td>Cell/Tissue culture samples were incompletely homogenized</td>
<td>Repeat the sample homogenization, increasing the length and extent of homogenization step.</td>
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<td></td>
<td>Samples used after multiple freeze-thaw cycles</td>
<td>Aliquot and freeze samples if needed to use multiple times</td>
</tr>
<tr>
<td></td>
<td>Presence of interfering substance in the sample</td>
<td>If possible, dilute sample further</td>
</tr>
<tr>
<td></td>
<td>Use of old or inappropriately stored samples</td>
<td>Use fresh samples and store correctly until use</td>
</tr>
<tr>
<td>Lower/Higher Readings in Samples and Standards</td>
<td>Improperly thawed components</td>
<td>Thaw all components completely and mix gently before use</td>
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<tr>
<td></td>
<td>Use of expired kit or improperly stored reagents</td>
<td>Always check the expiration date and store the components appropriately</td>
</tr>
<tr>
<td></td>
<td>Allowing the reagents to sit for extended times on ice</td>
<td>Always prepare fresh Master Reaction Mix before use</td>
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<tr>
<td></td>
<td>Incorrect incubation times or temperatures</td>
<td>Refer to Technical Bulletin and verify correct incubation times and temperatures</td>
</tr>
<tr>
<td></td>
<td>Incorrect volumes used</td>
<td>Use calibrated pipettes and aliquot correctly</td>
</tr>
<tr>
<td>Non-linear Standard Curve</td>
<td>Use of partially thawed components</td>
<td>Thaw and resuspend all components before preparing the reaction mix</td>
</tr>
<tr>
<td></td>
<td>Pipetting errors in preparation of standards</td>
<td>Avoid pipetting small volumes</td>
</tr>
<tr>
<td></td>
<td>Pipetting errors in the reaction mix</td>
<td>Prepare a Master Reaction Mix whenever possible</td>
</tr>
<tr>
<td></td>
<td>Air bubbles formed in well</td>
<td>Pipette gently against the wall of the tubes</td>
</tr>
<tr>
<td></td>
<td>Standard stock is at incorrect concentration</td>
<td>Always refer to the dilutions in the Technical Bulletin</td>
</tr>
<tr>
<td></td>
<td>Calculation errors</td>
<td>Recheck calculations after referring to Technical Bulletin</td>
</tr>
<tr>
<td></td>
<td>Substituting reagents from older kits/lots</td>
<td>Use fresh components from the same kit</td>
</tr>
<tr>
<td>Unanticipated results</td>
<td>Samples measured at incorrect wavelength</td>
<td>Check the equipment and filter settings</td>
</tr>
<tr>
<td></td>
<td>Samples contain interfering substances</td>
<td>If possible, dilute sample further</td>
</tr>
<tr>
<td></td>
<td>Sample readings above/below the linear range</td>
<td>Deproteinize samples</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Concentrate or dilute samples so readings are in the linear range</td>
</tr>
</tbody>
</table>