G6PDH

Order Information
Cat. No. 1 7900 99 10 026
   1 x 20 mL + R2 4 x 5 mL
R3 1 x 40 mL
   2 x 0.5 mL TruCal G6PDH
Calibrator set with 2 different levels

Intended Use
Diagnostic reagent for quantitative in vitro determination of glucose-6-phosphate dehydrogenase (G6PDH) in whole blood on photometric systems.

Summary
Glucose-6-phosphate dehydrogenase (G6PDH) is a cytosolic enzyme that catalyzes the conversion of glucose-6-phosphate (G-6-P) to 6-phosphogluconolactone in the first step in the pentose phosphate pathway. The pentose phosphate pathway is the major source for the NADPH required for anabolic processes. NADPH is required as hydrogen donor for numerous reductive processes as well as for stability of catalase and preservation and regeneration of the reduced form of glutathione. Both, catalase and glutathione are crucial for cell detoxification and cell protection from oxidative stress. Since red blood cells lack any other source of NADPH and are solely dependent on G6PDH, the primary enzyme of the pentose phosphate pathway.

Glucose-6-phosphate dehydrogenase (G6PDH) deficiency is one of the most common human genetic enzymopathies. People with G6PDH deficiency are at risk of hemolytic anemia in states of oxidative stress, infections and after ingestion of certain drugs or fava beans. [1]

Method
Enzymatic UV (photometric) method

Principle
Glucose-6-phosphate dehydrogenase (G6PDH) catalyzes the first step in the pentose phosphate shunt, oxidizing glucose-6-phosphate (G-6-P) to 6-phosphogluconate (6-PG) and reducing NADP to NADPH.

The increase of absorbance of NADPH is proportional to the G6PDH concentration in the sample.

The reagent contains 6-PGDH (6-phosphogluconate-dehydrogenase) inhibitors which prevent the production of a second molar equivalent of NADPH by erythrocyte 6-phosphogluconate dehydrogenase.

Reagents
Components and Concentrations
R1: Good's buffer modified pH 7.65 > 20 mmol/L
R2: NADP > 0.19 mmol/L
R3: G-6-P pH 7.65 > 0.1 g/L

Storage Instruction and Reagent Stability
The reagents are stable up to the end of the indicated month of expiry, if stored at 2 – 8°C and contamination is avoided. Do not freeze the reagents.

Warnings and Precautions
1. In very rare cases, samples of patients with gammapathy might give falsified results. [2]
2. Reticulocytes have higher G6PDH levels than mature red cells; it is not recommended to run the assay after a severe hemolytic crisis, since G6PDH may appear falsely elevated.
3. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, the results should always be assessed with the patient’s medical history, clinical examinations and other findings.
4. For professional use only.

Waste Management
Refer to local legal requirements.

Reagent Preparation
Dissolve a vial of R2 with 5 mL of R1 reagent (working reagent), mix gently, and avoid foaming.
Stability: 5 days at 2 – 8°C
R3 is ready to use.
Allow the reagent to reach room temperature before use. Close immediately after handling.

Materials Required
General laboratory equipment

Specimen
Whole blood collected with EDTA, heparin or ACD (acid-citrate-dextrose).
Sample collection in compliance with CLSI (NCCLS) [3]

Sample Preparation
For sample preparation, DiaSys G6PDH Hemolyzing Solution Cat. No. 1 7900 99 10 113 is required.
Sample preparation:
Hemolyzing Solution 9 parts
Sample/Calibrator/Control 1 part
Mix gently, avoid foaming and assay immediately.

Attention
G6PDH activity is reported in units per gram hemoglobin [U/g Hb] therefore the hemoglobin concentration must be determined prior to performing the G6PDH assay.

Stability
Red cell G6PDH is stable in whole blood for 1 week at 2 – 8°C, but is unstable in red cell hemolysate. A precipitate may appear 20/30 minutes after dilution (see sample preparation with G6PDH Hemolyzing Solution), probably due to the biological variability of the patient’s sample. Freezing of blood is not recommended. [4,5]

Assay Procedure
Applications for automated analyzers are available on request.
Manual procedure may slightly differ from applications for automated systems. This assay is tested on a manual spectrophotometer and on Hitachi, Cobas and Mindray systems.

Wavelength 340 nm (334 – 365 nm)
Optical path 1 cm
Temperature 37°C
Measurement Against air or distilled water

<table>
<thead>
<tr>
<th>Component</th>
<th>Calibrator</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Level 1</td>
<td>Level 2</td>
</tr>
<tr>
<td>Working reagent</td>
<td>1000 µL</td>
<td>1000 µL</td>
</tr>
<tr>
<td>Calibration</td>
<td>10 µL</td>
<td>10 µL</td>
</tr>
<tr>
<td>Sample</td>
<td>10 µL</td>
<td></td>
</tr>
<tr>
<td>Mix gently and incubate for 10 min. at 37°C, then add:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R3</td>
<td>2000 µL</td>
<td>2000 µL</td>
</tr>
<tr>
<td>Mix gently, read absorbance (A1) after exactly 2 minutes, read absorbance (A2) again after 5 minutes.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Calculation
Manual calculation of G6PDH activity (U/L – 37°C)

ΔA Calibrator level 1 = A2 calibrator level 1 – A1 calibrator level 1
ΔA Calibrator level 2 = A2 calibrator level 2 – A1 calibrator level 2
ΔA Sample = A2 sample – A1 sample
Calculate \( \Delta A/ \text{minute} (\Delta A/\text{min}) = (A2 - A1) / 5 \)

G6PDH (U/L, 37°C) =

\[ \Delta A/ \text{min} \times \text{(Total volume/Sample volume)} \times (1/\varepsilon \, d) \times 1000 \]

\( \varepsilon = 6.3 \) = millimolar absorption of NADPH at 340 nm

\( d = 1 \, \text{cm} \) = optical path length

1000 = Factor to convert activity to Liter

G6PDH (U/L, 37°C) =

\[ \Delta A/ \text{min} \times (3.01/0.01) \times (1/6.3) \times 1000 \]

\( \Delta A/ \text{min} \times (301000) / 6.3 \)

\( \Delta A/ \text{min} \times 47778 \)

Manual calculation of G6PDH activity (U/g Hemoglobin at 37°C)

Considering the value of total hemoglobin (Total Hb) of each sample [g/dL], apply the subsequent formula:

\[ \text{G6PDH (U/g Hb)} = \frac{\text{G6PDH (U/L, 37°C)}}{\text{Total Hb (g/dL) \times 10}} \]

Calibrators and Controls

DiaSys TruCal G6PDH calibrator is recommended. TruCal G6PDH calibrator values have been made traceable to a commercially available test. Use DiaSys TruLab G6PDH for internal quality control. Each laboratory should establish corrective action in case of deviations in control recovery.

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Kit size</th>
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<tbody>
<tr>
<td>1 7900 99 10 045</td>
<td>3 x 0.5 mL</td>
</tr>
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</table>

Performance Characteristics

Data evaluated on MINDRAY BS300

Exemplary data mentioned may slightly differ in case of deviating measurement conditions.

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Measuring range up to 3200 U/L
When values exceed this range, use half sample volume and multiply the result by 2.

Limit of detection* = 29 U/L
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*lowest measurable concentration which can be distinguished from zero; mean + 3 SD (n = 20) of an analyte free specimen.

Interfering substance | Interferences ≤ 10% up to
Copper | Strong inhibitor
Sulphate | Strong inhibitor
Ascorbic acid | 50 mg/dL
Bilirubin (total) | 40 mg/dL
Lipemia (Intralipid®) (triglycerides) | 4000 mg/dL

For further information on interfering substances refer to Young DS. [6]

Precision

<table>
<thead>
<tr>
<th></th>
<th>Sample 1</th>
<th>Sample 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean [U/L]</td>
<td>191</td>
<td>1374</td>
</tr>
<tr>
<td>CV [%]</td>
<td>1.4</td>
<td>0.7</td>
</tr>
</tbody>
</table>

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<tr>
<td>CV [%]</td>
<td>1.7</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Method comparison (n=21)

<table>
<thead>
<tr>
<th>Test x</th>
<th>Competitor G6PDH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test y</td>
<td>DiaSys G6PDH</td>
</tr>
<tr>
<td>Slope</td>
<td>0.988</td>
</tr>
<tr>
<td>Intercept</td>
<td>–13 U/L</td>
</tr>
<tr>
<td>Coefficient of correlation</td>
<td>( r = 0.991 )</td>
</tr>
</tbody>
</table>

Conversion Factor

G6PDH (U/L) \times 0.0167 = G6PDH [ukat/L]

Reference Range [7]

Adults: 7.9 – 16.3 Ug/g Hb

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

Literature


DiaSys Diagnostic Systems GmbH
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Germany
www.diasys-diagnostics.com