Intended Use
For the quantitative, kinetic determination of glucose-6-phosphate dehydrogenase (G6PD) in blood at 340 nm. For in vitro diagnostic use only.

Clinical Significance
G6PD assays are most commonly performed to determine deficiency of G6PD, which is widely prevalent throughout the world. It has been determined that G6PD deficiency in red cells is the basis for certain drug-induced hemolytic anemias. This type of susceptibility to drug-induced hemolysis is often called “primaquine sensitivity” because studies which led to its characterization were made during investigations of the hemolytic properties of this antimalarial compound.

Summary
Glucose-6-phosphate dehydrogenase (G6PD, D-glucose-6-phosphate: oxidoreductase, EC 1.1.1.49) 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. This procedure is a modification of the spectrophotometric methods of Kornberg and Horecker2 and of Lohr and Waller3, involving the following reaction:

\[ \text{G-6-P} + \text{NADP}^+ \rightarrow 6\text{-PG} + \text{NADPH} + \text{H}^+ \]

Nicotinamide adenine dinucleotide phosphate (NADP) is reduced by G6PD in the presence of G-6-P. The rate of formation of NADPH is proportional to the G6PD activity and is measured spectrophotometrically as an increase in absorbance at 340nm. Production of a second molar equivalent of NADPH results are best reported in terms of hemoglobin concentration.

Reagents
G6PD R1 Reagent: Reconstituted reagent will contain NADP, 1.5 mM, and maleimide, 12 mM. Also contains buffer, stabilizer and lysing agent.
G6PD R2 Reagent: Glucose-6-Phosphate, 1.05 mM, buffer and magnesium salt. Sodium azide added as preservative.

Precautions
1. These reagents are for in vitro diagnostic use only.
2. Normal precautions exercised in handling laboratory reagents should be followed. Dispose of waste observing all local, state and federal laws.
3. R1 Reagent is HARMFUL. May cause sensitization by inhalation and skin contact. Wear suitable protective clothing.
4. R2 Reagent contains sodium azide which may react with lead and copper plumbing to form highly explosive metal azides. Avoid azide accumulation.

Reagent Preparation
1. R1 reagent is prepared by reconstituting with the volume of deionized water indicated on vial label or application sheet. Swirl gently and invert several times to dissolve contents. Wait 5 minutes and mix again. NOTE: For manual use, see reagent preparation instructions listed in “MANUAL PROCEDURE” section.
2. R2 reagent is supplied ready to use.
3. Once 6.0ml R1 reagent has been properly reconstituted add 12ml of R2 reagent to the R1 reagent (1 to 2 ratio). The R1/ R2 mixture will be the working reagent.

Storage and Stability
Manual Procedure

Prepare working R1 reagent by adding lyse as the diluent instead of DH2O. Add volume stated on the R1 vial. This reagent can now be used as stated below. **NOTE:** Do not use DH2O to reconstitute the R1 vial for manual procedure.

The temperature of the reaction must be maintained at 37°C or some other constant temperature (see “Temperature Correction” section).

1. Prepare reaction mixture:
   a. To a labeled cuvette, add 1.0 ml R1 Reagent.
   b. Add 0.01 ml blood and mix thoroughly to completely suspend erythrocytes. Let stand at room temperature (18-26°C) for 5-10 minutes.
   c. Add 2.0 ml R2 Reagent and mix gently by inverting several times. Proceed to step 2.

2. Place cuvette in constant temperature cuvette compartment or water bath and incubate for approximately 5 minutes.

3. Read and record absorbance (A1) of TE buffer and incubate for approximately 5 minutes.

4. Exactly 5 minutes later, read and record absorbance (A2).

5. To determine G6PD activity, refer to “Calculations” section.

Calibration

The procedure is standardized on the basis of the millimolar absorptivity of NADPH, which is 6.22 at 340 nm. Measurement of the rate of increase in absorbance (ΔA) at 340 nm serves to quantitate enzymatic activity.

Quality Control

Reliability of test results should be monitored by use of control materials with known values within each run. Pointe Scientific glucose-6-phosphatase dehydrogenase controls are suitable for this purpose. (Catalog number G7583-CTL) It is recommended that each laboratory establish its own frequency of control determination.

Calculations

\[ \Delta A \text{ per min} = \frac{A2 - A1}{5} \]

G6PD activity can be expressed as either U/g hemoglobin (Hb) or as U/10\(^{12}\) erythrocytes (RBC).

\[ \text{G6PD (U/g Hb)} = \Delta A \text{ per min} \times \frac{100 \times 3.01}{0.01 \times 6.22 \times \text{Hb (g/dl)}} \times \text{TCF} \]

or

\[ \text{G6PD (U/10}^{12}\text{RBC)} = \Delta A \text{ per min} \times \frac{3.01 \times 10^{12} \times \text{TCF}}{0.01 \times 6.22 \times (N \times 10^6) \times 1000} \]

Where:

- 100 = Factor to convert activity to 100 ml
- 3.01 = Total reaction volume (ml)
- 0.01 = Sample volume (ml)
- 6.22 = Millimolar absorptivity of NADPH at 340 nm
- Hb (g/dl) = Hemoglobin concentration for each specimen
- TCF = Temperature Correction Factor (1 at 37°C)

This equation reduces to:

\[ \text{G6PD (U/10}^{12}\text{RBC)} = \Delta A \text{ per min} \times \frac{48.390}{N} \times \text{TCF} \]

Where: N = red cell count divided by 10\(^6\)

* TCF= temperature correction factor (1 at 37°C)

** Additional temperature correction factors are available upon request.

Example:

Assay of a specimen which had a red cell count of 4.6 x 10\(^6\)/mm\(^3\) and a hemoglobin concentration 15.2 g/dl resulted in a ΔA per min at 37°C of 0.028.

\[ \text{G6PD (U/g Hb)} = 0.028 \times 48.390 = 8.9 \]

\[ \text{G6PD (U/10}^{12}\text{RBC)} = 0.028 \times 48.390 = 295 \]

**NOTE:** If ΔA per min is greater than 0.060, repeat determination using 5 μl blood and multiply results by 2.

Use of Buffy-Coat-Free Sample

Under normal circumstances G6PD activity contributed by leukocytes, platelets and serum is relatively small. However, as reported by Echler and others, more accurate measurement of red cell G6PD activity, especially in the presence of anemia and/or leukocytosis, can be achieved by using buffy coat-free blood samples for assay. Thus, in case of a borderline value obtained with whole blood, it may be warranted to repeat the assay on a buffy coat-free sample.

Temperature Correction

When temperature is 37°C, no temperature correction factor (TCF) is required in the calculations. If assay is performed at another temperature a TCF must be used.

<table>
<thead>
<tr>
<th>Cuvette Temperature</th>
<th>TCF</th>
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</thead>
<tbody>
<tr>
<td>25°C</td>
<td>1.98</td>
</tr>
<tr>
<td>30°C</td>
<td>1.37</td>
</tr>
</tbody>
</table>

Unit Definition

One International Unit (U) is that amount of G6PD activity that will convert 1 micromole of substrate per minute under the conditions specified in this insert.

**Expected Values**

A recommended reference range for G6PD measured at 37°C is:

\[ 12.1 \pm 2.09 \text{ U/g Hb} \]
\[ 351 \pm 60.8 \text{ U/10}^{12}\text{ RBC} \]

Values for newborns may range somewhat higher. It is highly recommended that each laboratory establish its own expected range.

Performance Characteristics

**Assay Range:** The maximum G6PD activity which may be measured by this procedure is approximately 21.0 U/g Hb or 609 U/10\(^{12}\) RBC.

<table>
<thead>
<tr>
<th>Observed Data</th>
<th>Theoretical Result</th>
<th>Percent Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.78 U/g Hb</td>
<td>2.78 U/g Hb</td>
<td>100.0%</td>
</tr>
<tr>
<td>5.29 U/g Hb</td>
<td>5.56 U/g Hb</td>
<td>95.1%</td>
</tr>
</tbody>
</table>
10.80 U/g Hb  |  11.12 U/g Hb  |  97.1%
20.69 U/g Hb  |  22.24 U/g Hb  |  93.0%

**Precision:** Precision studies were performed on a Roche Cobas Mira following the guidelines contained in NCCLS document EP5-T2. The data is presented in units that an automated analyzer will produce for G6PD activity (U/L). It is highly recommended that precision of the assay be verified on each analyzer before use.

<table>
<thead>
<tr>
<th>Within Day (n=20)</th>
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</thead>
<tbody>
<tr>
<td>Mean</td>
<td>S.D.</td>
<td>C.V.</td>
</tr>
<tr>
<td>257</td>
<td>23.7</td>
<td>9.2%</td>
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<tr>
<td>685</td>
<td>18.3</td>
<td>2.8%</td>
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<tr>
<td>1939</td>
<td>48.0</td>
<td>2.5%</td>
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</table>

<table>
<thead>
<tr>
<th>Day to Day (n=20)</th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>S.D.</td>
<td>C.V.</td>
</tr>
<tr>
<td>269</td>
<td>30.8</td>
<td>11.4%</td>
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<tr>
<td>700</td>
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<td>4.1%</td>
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<tr>
<td>2014</td>
<td>43.0</td>
<td>2.1%</td>
</tr>
</tbody>
</table>

**Sensitivity:** Assuming the limit of sensitivity to represent a change in absorbance at 340nm of 0.001 per minute, a G6PD activity of 0.4 U/g Hb or 11 U/10^12 RBC may be detected using this procedure (assuming a hemoglobin concentration of 12.0 g/dL and a red cell count of 4.5 x 10^12/mm^3).

**Specificity:** The oxidation of glucose-6-phosphate by G6PD is specific. Any non-specific formation of NADPH due to oxidation of other substrates by endogenous enzymes occurs during the preincubation period. 6-Phosphogluconate dehydrogenase is completely inhibited by maleimide in the reagent system.

**Correlation:** A comparison study between the Pointe Scientific method and that of Sigma Diagnostics yielded a linear regression equation with $y = 0.97x + 0.07$ and a correlation coefficient of 0.994.

**References**