Glucose-6-Phosphate dehydrogenase (G-6-PDH) Quantitative assay

**REF: 252 001** 10 tests
Reagent 1a: G6PDH Reagent 2 x 5.5 ml (Lyophilisates) 12 ml
Reagent 2: Starter Reagent 20 ml

**REF: 252 002** 50 tests
Reagent 1a: G6PDH Reagent 2 x 5.5 ml (Lyophilisates) 60 ml
Reagent 2: Starter Reagent 2 x 5.30 ml

**Intended Use**
Spectrum-Diagnostics G-6-PDH reagent is intended for the in-vitro quantitative UV diagnostic estimation of G-6-PDH in RBC's.

**Background**
Glucose-6-Phosphate Dehydrogenase (G6PDH) deficiency is one of the most common human enzyme deficiencies in the world. During G6PD deficiency, the red cells are unable to regenerate reduced Nicotinamide adenine dinucleotide phosphate (NADPH); a reaction that is normally catalyzed by the G6PD enzyme. Since the X chromosome carries the gene for G6PD enzyme, this deficiency mostly affects the males. The two major conditions associated with G6PD deficiency are hemolytic anemias and neonatal jaundice, which may result in neurological complications and death. Screening and detection of G6PD deficiency helps in reducing such episodes, through appropriate selection of treatment, patient counseling and abstinence from disease precipitating drugs such as anti malarials and other agents.

**Method**
UV-Kinetic Method.

**Assay Principle**
G6PDH in the RBC's is released by a lysing agent present in the reagent. The G6PDH released catalyzes the oxidation of Glucose 6-phosphate with the reduction of NADP to NADPH. The rate of reduction of NADP to NADPH is measured as an increase in absorbance which is proportional to the G6PDH activity in the sample.

G-6-P + NADP \rightarrow \text{Glucose-6-P + NADPH + H}^+

**Reagents**
- Reagent 1a (R1a): G6PDH Reagent
- Reagent 1b (R1b): Diluent Reagent
- Reagent 2 (R2): Starter Reagent

**Precautions and Warnings**
Do not ingest or inhale. In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.

**Reagent Storage and Stability**
Reagents are ready-to-use. When stored at 2 – 8 °C; they are stable up to the expiry date stated on the label. Reconstituted G6PDH Assay solution is stable for 8hrs at room temp. (15 - 25 °C) or 5 days refrigerated (2 - 8 °C).

**Sample collection and preparation**
Whole blood collected in EDTA, Heparin or ACD is satisfactory. Red cell G-6-PDH is stable in whole blood for one week refrigerated (2-8 °C), but is unstable in red cell hemolysates. Freezing of blood is not recommended. Since activity is reported in terms of number of red cells or grams of hemoglobin. The red cell count or hemoglobin concentration should be determined prior to performing the G-6-PDH assay.

The integrity of erythrocytes collected in ACD is preserved even after prolonged storage so that obtaining accurate red cell counts usually poses no problem. However, red cell counts on specimens collected in heparin become unreliable after about 2 days. Thus, for heparinized samples, results are best reported in terms of hemoglobin concentration. Both copper, which completely inhibits the enzyme at a concentration of 100 μmol/L, and sulfate ions (0.005 mol/L) will decrease observed values of G-6-PDH activity. Certain drugs and other substances are known to influence circulating levels of G-6-PDH. Reticuloocytes have higher G-6-PDH levels than mature red cells. Therefore it is not recommended that assays be performed after a severe hemolytic crisis, since G-6-PDH levels appear falsely elevated. Under those conditions, detection of deficiency may require family studies. Testing may be more helpful after the level of mature red cells has returned to normal. Under normal circumstances, activity contributed by leucocytes, platelets and serum is relatively small. However, in cases of extreme anemia, grossly elevated white counts or very low levels of red cell G-6-PDH activity, the contribution to the total made under these conditions may be significant. See “Use of Buffy-Coat-Free Samples” section.

**Reagent preparation**

G6PDH Reagent Preparation
Is prepared by reconstituting G6PDH Reagent with the volume of diluent as stated on the vial. Swirl gently and invert several times to dissolve the contents. Wait 2-3 minutes and mix again.

**G-6-PDH Starter Reagent**
Is supplied ready to use.

**System Parameters**
- Wavelength: 340 nm
- Optical path: 1 cm
- Assay type: UV-Kinetic
- Direction: Increase
- Sample volume: 10 μl
- Reagent ratio: 3 ml
- Temperature: 30 or 37 °C
- Measurement: Against distilled water
- Delay/Lag/Time: 300 sec
- Interval Time: 60 sec
- NO. OF READINGS: 05
- Blank Absorbance Limit: < 0.8
- Factor: 4839

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

1. Prepare reaction mixture:
   a) Add 0.01 ml blood to 1.0 ml of G-6-PDH working solution and mix thoroughly to completely suspend erythrocytes. Let stand at room temperature (18-26°C) for 5-10 minutes.
   b) Add 2.0 ml Starter Reagent directly to vial and mix gently by inverting several times.
   c) Transfer contents of vial to cuvette labeled Test & proceed with Step 2.
Place cuvette in constant temperature cuvette compartment or water bath and incubate for approximately 5 minutes to obtain thermal equilibrium.

Read and record absorbance (A) of Test at 340nm vs water or Potassium Dichromate solution. This is INITIAL A. (If using the water bath or incubator, return the cuvet to it)

Exactly 5 minutes later, again read and record absorbance. This is FINAL A.

To determine G-6-PDH activity, refer to “calculations” section.

\[ \Delta A/\text{min} = \frac{\text{Final A} - \text{Initial A}}{5} \]

\[ \text{G6PDH (U/g Hb)} = \Delta A/\text{min} \times \frac{100 \times 3.01}{0.01 \times 6.22 \times \text{Hb (g/dl)}} \times \text{TCF} \]

\[ \text{G6PDH (U/g Hb)} = \Delta A \times \frac{4839}{\text{Hb (g/dl)}} \times \text{TCF} \]

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

\[ \text{G6PDH (U/10^{12} RBC’s)} = \Delta A \times \frac{48390}{\text{RBC count in million}} \]

Note: If \( \Delta A \) is greater than 0.060, repeat the assay using 5 µL blood and multiply results by 2.

**TEMPERATURE CORRECTION FACTOR**

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<th>Assay Temperature</th>
<th>Desired Reporting Temperature</th>
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<td>25°C</td>
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<tr>
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**CALIBRATION**

The procedure is standardized on the basis of the millimolar absorptivity of NADPH, which is 6.22 at 340nm. The oxidative conversion of G-6-P by G-6-PDH leads to reduction of NADP to NADPH on a molar equivalent basis. Measurement of the rate of increase in absorbance (ΔA) at340nm serves to quantitate enzymatic activity. The maximum G-6-PDH activity which may be measured is 650 U/10¹² RBC or 19.5 U/g Hb.

**USE OF BUFFY-COAT-FREE SAMPLE**

Under normal circumstances G-6-PDH activity contributed by leucocytes, platelets and serum is relatively small. However, as reported by Echler and others, more accurate measurement of G-6-PDH activity, specially in the presence of anaemia and/or leucocytosis, 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.

**Expected Values**

G6PDH Activity (U/g Hb): 4.6 -13.5 at 30°C

(U/10¹² RBC’s): 146 - 376 at 30°C

202 - 522 at 37°C

**Note:** It is recommended for each laboratory to establish and maintain its own reference values. The given data are only an indication.

**Performance Characteristics**

**Precision:**

Precision studies were performed on a Roche Cobas Mira following the guidelines contained in NCCLS document EP5-T2.15

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.

**With day (n=20)**

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<th>Mean</th>
<th>SD</th>
<th>CV%</th>
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**Day to day (n=20)**

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**Sensitivity**

G6PD activity of 0.4 U/g Hb or 11 U/10¹² RBC may be detected using this procedure (assuming a hemoglobin concentration of 12.0 g/dl and a red cell count of 4.5 x10⁶/mm3).

**Linearity**

The assay is linear up to 19.5 µg Hb.

**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 Spectrum method and that of Sigma Diagnostics yielded a linear regression equation with y = 0.968 x + 0.068 and a correlation coefficient of 0.994.

**Waste Disposal**

This product is made to be used in professional laboratories. Please consult local regulations for a correct waste disposal.

**References**