Glucose-6-Phosphate dehydrogenase (G-6-PDH) Single Assay vials

REF: 253 001 (10 x 1.1 ml) 10 tests

Reagent 1a: G6PDH Assay vials 10 x 1.1 ml
Reagent 1b: Assay Diluents 12 ml
Reagent 2 : Substrate Solution 22 ml

Intended Use

Spectrum-Diagnostics G-6-PDH reagent is intended for the in-vitro quantitative UV diagnostic estimation of G-6-PDH in human serum.

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 releases 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 → Gluconate -6-P+ NADPH+H⁺

Reagents

Reagent 1a (R1a): G6PDH Assay vials
Reagent 1b (R1b): Assay Diluents
Reagent 2 (R2): Substrate Solution

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 and standard are ready-to-use. When stored at 2 – 8 °C; they are stable up to the expiry date stated on the label. Recomposed 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.

Reagent preparation

G-6-PDH ASSAY SOLUTION Preparation

Is prepared by reconstituting G-6-PDH assay vials with the volume of Assay 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 SUBSTRATE SOLUTION

Is supplied ready to use.

System Parameters

Wavelength 340 nm
Optical path 1 cm
Assay type UV-Kinetic
Direction Increase
Sample: Reagent Ratio 1:100
Temperature 30 °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
Low Normal at 30°C 4.6 µg/g Hb
High Normal at 30°C 13.5 µg/g Hb
Linearity at 30°C 19.5 µg/g Hb

Procedure

The temperature of the reaction mixture should be maintained at 30°C or some other constant temperature (see “Temperature Correction” section).

1. Prepare reaction mixture :

a) Add 0.01ml blood to 1.0 ml of G-6-PDH Assay solution and mix thoroughly to completely suspend erythrocytes. Let stand at room temperature (18-26°C) for 5-10 minutes.

b) Add 2.0ml G-6-PDH Substrate solution directly to vial and mix gently by inverting several times.

c) Transfer contents of vial to cuvette labeled Test & proceed with Step 2.
2. Place cuvet in constant temperature cuvet compartment or water bath and incubate for approximately 5 minutes to obtain thermal equilibrium.

3. 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)

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

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

**CALCULATION**

\[
\text{A per min} = \text{FINAL A} - \text{INITIAL A}
\]

G-6-PDH activity is expressed as U/1012 erythrocytes (RBC) or U/g hemoglobin (Hb).

\[
\text{G-6-PDH (U/1012RBC)} = \frac{\text{A per min} \times 48,390 \times \text{TCF}}{N}
\]

Where:

\[
N = \text{Red cell count divided by 106}
\]

\[
\text{TCF} = \text{Temperature correction factor (1 at 30°C)}
\]

G-6-PDH (U/g Hb) = \( \text{A per min} \times 4839 \times \text{TCF} \)

\[
\text{Hb(g/dL)}
\]

**EXAMPLE:**

Assay of a specimen which had a red cell count of 4.6 x 10^6/mm3 and a hemoglobin concentration 15.2 g/dL resulted in a A per min at 30°C of 0.026.

G-6-PDH(U/1012RBC) = 0.026 x 48,390 = 295

G-6-PDH(U/g Hb) = 0.026 x 4839 = 12

**Note:** If A per min is greater than 0.060, repeat determination using 5ìL blood and multiply results by 2

**CALIBRATION**

The procedure is standardized on the basis of the milimolar 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 (A) at340nm serves to quantitate enzymatic activity. The maximum G-6-PDH activity which may be measured by this procedure is approximately 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.

**TEMPERATURE CORRECTION**

When temperature of 30°C, no temperature correction factor (TCF) is required in the calculations. If assay is performed at a room temperature other than 30°C, a TCF must be used. When the temperature is 37°C, the TCF is 0.66.

**Linearity**

The assay is linear up to 19.5 µg Hb

**Expected Values**

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

(100 - RBC): 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.

**Spectrum Diagnostics does not interpret the results of a clinical laboratory procedure; interpretation of the results is considered the responsibility of qualified medical personnel. All indications of clinical significance are supported by literature references.**

**Waste Disposal**

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

**References**


**ORDERING INFORMATION**

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<th>CATALOG NO.</th>
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