SPECTRUM

The Creative Approach to Bioscience

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

REF: 252 001 10 tests

Reagent 1a: G6PDH Reagent	2 x 5.5 ml (Lyo-vials)
Reagent 1b: Diluent Reagent	12 ml
Reagent 2 : Starter Reagent	20 ml

REF: 252 002 50 tests

Reagent 1a: G6PDH Reagent	10 x 5.5 ml (Lyo-vials)
Reagent 1b: Diluent	60 ml
Reagent 2 : Starter Reagent	2 x50 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 Nicotineamide 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 anaemias 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.

$$\text{G-6-P} + \text{NADP} \xrightarrow{\text{G-6-PDH}} \text{Gluconate-6-P} + \text{NADPH} + \text{H}$$

Reagents

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

Precautions and Warnings

Do not ingest or inhalate. 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 ^{o}C; they are stable up to the expiry date stated on the label.
Recontituted 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.

SYMBOLS IN PRODUCT LABELLING

EC REP Authorised Representative	Use by/Expiration Date
IVD For in-vitro diagnostic use	CAUTION. Consult instructions for use
LOT Batch Code/Lot number	
REF Catalogue Number	Manufactured by
Consult instructions for use	(Xi) - Irritant
Temperature Limitation	

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 100ìmol/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. Reticulocytes 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
Samplevolume	10 µλ
Reagent Ratio	3 ml
Temperature	30 or 37 ^{o}C
Measurement	Against distilled water
Delay/Lag/Time	300 sec
Interval Time	60 sec
NO. OF READINGS	05
Blank Absobance 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 0.01ml 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 2.0ml Starter Reagent directly to vial and mix gently by inverting several times.

c)Transfer contents of vial to cuvette labeled Test & proceed with Step2.

2.Place cuvett in constant temperature cuvett 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

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

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

$$\textbf{G6PDH (U/g Hb)} = \Delta A \times \frac{4839}{Hb\ (g/dl)} \times TCF$$

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

$$\textbf{G6PDH (}U/10^{12}\ RBC's\textbf{)} = \Delta A \times \frac{48\ 390}{RBC\ count\ in\ milion}$$

Note: If ΔA is greater than 0.060, repeat the assay using 5 µL blood and multiply results by 2

TEMPERATURE CORRECTION FACTOR

Assay Temperature	Desired Reporting Temperature		
	25°C	30°C	37°C
25°C	1.00	1.32	1.82
30°C	0.76	1.00	1.39
37°C	0.55	0.72	1.00

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 (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 boderline 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
6.4 -18.7 at 37°C
($U/10^{12}$ 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)

Mean	SD	CV%
262	23.6	9.01 %
656	18.4	2.8 %
1941	48.2	2.48 %

Day to day (n=20)

Mean	SD	CV%
268	31.1	11.6 %
689	28.8	4.18 %
2012	42.9	2.13 %

Sensitivity

G6PD activity of 0.4 U/g Hb or11 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 U/g 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.
S56: dispose of this material and its container at hazardous or special waste collection point.
S57: use appropriate container to avoid environmental contamination.
S61: avoid release in environment. refer to special instructions/safety data sheets.

References

S.K. Sood et al., The Indian journal of path and micro,, 24 (1981), 89. Lubin, B.H. and Oski, F.A., J. Pediatr. 70 (1967), 788.

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