OBio...
Supporting Care to Cure
A Division of Orchard Medical

Glucose-6-phosphate dehydrogenase (G-6-PDH)

IVD

Order Information

Cat. No.
OAR1091

Kit Configuration

R1: 2 x 15 mL
R2: 1 x 10 mL

Summary

G-6PDH deficiency is one of the most common human enzyme deficiencies, and it is estimated to affect more than 400 million people worldwide. Although most of the enzyme-deficient subjects are asymptomatic, deficient individuals may show episodic haemolytic anemia induced by infections or certain drugs and a spontaneous chronic non spherocytic haemolytic anemia.

Method

Enzymatic UV Method

Principle

The enzyme activity is determined by measurement of the rate of absorbance change at 340 nm due to the reduction of NADP+.

Reagent Storage Instructions and Stability

R1 – Lysing reagent
R2 – Buffer Solution
R3 – Substrate Solution

Composition

Reagent: Triethanolamine-31.7 mmol/L, EDTA-3.2 mmol/L, NADP-0.34 mmol/L, Glucose-6-phospate-0.58 mmol/L

Warnings and Precautions

1. **In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.**
2. **Wear suitable gloves and eye/face protection.**
3. **Always use safety pipettes to pull the reagents into a pipette.**
4. **Reagents may contain some non-reactive and preservative components. It is suggested to handle carefully, avoid direct contact with skin and do not swallow.**
5. **Do not swallow. Avoid contact with skin and mucous membrane.**
6. **For professional use only!**

Waste Management

Please refer to local legal requirements.

Reagent Preparation

The reagent and the standard are ready to use.

Materials required but not provided

NaCl solution 9 g/L
General laboratory equipment

Specimen

Serum, heparin plasma or EDTA blood Stability:
7 days at 2° – 8 °C,
Only freeze once!

Assay Procedure

Wavelength	**340 nm**
Light path	**10mm**
Temperature	**37°C**
Blank	**Against Water Blank**

1. **Take 1 mL of R1 and 0.01 mL (10 µL) of whole blood mix it and keep at room temperature for 10 minutes, to prepare hemolysates or sample.**
2. **Take 0.5 mL of R2 mix with 0.5 ml of R3 to prepare working reagent and add 0.5 mL of whole blood hemolysates prepared in step 1.**

	Sample
Working Reagent	**1000 µL**
Sample	**500 µL**
Mix, Incubate for 1 min. and read absorbance after every 1 min. for 3 min.	

Calculation:

ΔA/min and multiply by the corresponding factor from table below:

$$\text{G6PDH activity (U/g Hb)} = \Delta A/\text{min} \times \frac{4839}{\text{Hb (g/dL)}}$$

$$\text{G6PDH activity (U/10}^{12}\text{ RBCs)} = \Delta A/\text{min} \times \frac{48390}{\text{RBC Count in Million}}$$

Quality Control

For internal quality normal and abnormal controls should be assayed with each batch of samples.
Each laboratory should establish corrective action in case of deviations in control recovery.

Performance Characteristics Measuring Range

Measuring range of the assay is 0.5 to 22 U/g Hb.

Specificity/Interferences

No interference was observed by, Ascorbic acid upto 30 mg/dL, and Triglycerides upto 2000 mg/dL.

Sensitivity/Limit of Detection

The lower limit of detection is 0.5 U/g Hb.

Linearity

The higher limit of detection is 22 U/g Hb.

Precision

Intra assay n = 20	Mean [U/g Hb]	SD [U/g Hb]	CV [%]
Sample 1	4.73	0.24	5.05
Sample 2	12.58	0.37	2.91

Inter assay n = 20	Mean [U/g Hb]	SD [U/g Hb]	CV [%]
Sample 1	8.63	0.18	2.03
Sample 2	14.38	0.28	1.98

Method Comparison

A comparison of Nucleus Diagnosys G6PDH kit (y) with a commercially available test (x) using 20 samples gave following results:
$y = 1.006x + 0.102$; $r^2 = 0.988$.

Reference Range

G6PDH activity (U/g Hb): 4.6 to 14.5 at 30˚C / 6.4 to 20.5 at 37˚C
(U/10^{12} RBCs) : 146 to 416 at 30˚C / 202 to 575 at 37˚C

The activity of G6PDH is reported in HB. Concentration or RBC count the same should be determined before performing the assay. RBCs are well preserved in ACD and such samples give accurate count, heparin samples becomes unreliable after 2 days and in such cases results are best reported in Hb concentration. Copper and Sulphate ions inhibit the G6PDH activity, hence handle carefully and use washed tips and clean vial as well as tips. In cases of severe anemia, leucocytosis or very low G6PDH levels, use the sample after removing the Buffy coat is recommended.
Each laboratory should check if reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

Literature

1. **Tietz Textbook of Clinical Chemistry. Philadelphia: W.B Saunders Company; 1986.p.1501.12**
2. **Diagnostic Hematology by Rodak, W.B Saunders, 1995 Ed: 218.**
3. **Varley H. Practical Clinical Biochemistry, 5th Edition, 713-729.**
4. **WHO, Tech. Report. Serial No: 366, 1967.**

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