

G-6-PDH

(Quantitative Enzymatic Determination of Glucose-6-phosphate Dehydrogenase in Serum, Plasma or Erythrocytes)

Cat.No.	Package Size		
	R1a	R1b	R2
143 000	20 x for 1.0 mL	1 x 20 mL	1 x 40 mL

TEST PRINCIPLE

G6PDH is an enzyme of the Hexose-Monophosphate Pathway, the only one related to primary blood disorders. It catalyzes the reaction of Glucose-6-P to Phosphogluconate in the RBC. G6PDH presents several genetic variants. The strong reduction of G6PDH activity in a few genetic variants may cause mild to severe hemolytic disease and crisis, sometimes with fatal outcome. G6PDH 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. The increase of absorbance of NADPH, by reduction of NADP, is proportional to the activity of the G-6-PDH in the sample and is measured kinetically.

REAGENTS

Components and concentrations (in the test) :

Good's Buffer	>20 mmol/L
G-6-P	>0.1 g/L
NADP	>0.19 mmol/L
Activators, Stabilizers	

Stability:

Reagents, stored at 2-8°C, are stable up to the expiry date printed on the labels

Preparation of Working Reagent R1

Add 1 ml of Reagent 1b to one vial of Reagent 1a. Mix gently to dissolve. **STABILITY: 5 days at 2-8°C.**

Reagent R2 is ready to use

Close vials immediately after use, avoid any contamination!

SAMPLES

- Fresh serum or plasma (EDTA/heparine) free of hemolyses
- Whole blood collected with EDTA, heparine or ACD (Acid-Citrate-Dextrose).

Red cell G6PDH is stable in whole blood for 1 week at 2-8°C, but unstable in Red Cell hemolysate. Freezing of blood is not recommended (2,4)

ASSAY PROCEDURE Serum/Plasma

- Wavelength: 340 nm (334-365 nm)
- Cuvette: 1 cm
- Reading: against air or dist. water
- Temperature: 37°C

Let reagent reach working temperature

Pipette into test tubes or cuvettes:

S = Sample, Cal = Calibrator, Con = Control :

	Cal/Con	S
Working Reagent R1	1000 µL	1000 µL
Calibrator/Control	10 µL	----
Sample	----	10 µL

Mix well and incubate 5-10 min at 37°C, then add

Reagent 2	2000 µL	2000 µL
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Mix well, and after 2 min read A₁ of Calibrator/Controls and Samples.

Repeat readings exactly 5 min later -> A₂.

Determine absorbance/min (ΔA/min) for Calibrator, Control and Samples, calculating

$$\frac{(\text{SECOND readings} - \text{FIRST readings})}{5}$$

CALCULATION

$$\text{G6PDH (U/L 37°C)} = \text{Cal. Value} \times \frac{\Delta\text{As/min}}{\Delta\text{A}_{\text{CAL}}/\text{min}}$$

CALIBRATION AND QUALITY CONTROL

For calibration and control use adequate materials available from Greiner.

REFERENCE VALUES (37°C)

0,00 – 0,18 U/L

It is recommended that each laboratory determines its own reference values.

ASSAY PROCEDURE Erythrocytes

See special procedures for Analyzers and note the use of Lysing Reagent – see Note 7

Before testing G6PDH determine (A) or (B) :

- (A) number of erythrocytes (RBC) expressing G-6-PDH activity as **U/10¹² erythrocytes (RBC)**, or
- (B) conc. of Hemoglobin in g/dL, expressing G-6-PDH activity as **U/g of Hemoglobin**

The most accurate red cell counts are received with erythrocytes in ADC, due to increased stability of erythrocytes in that medium.

For heparin samples best results are reported in terms of hemoglobin concentration.

- Wavelength: 340 nm (334-365 nm)
- Cuvette: 1 cm
- Reading: against air or dist. water
- Temperature: 37°C

Let reagent reach working temperature

Pipette into test tube or cuvette :

	S
Working Reagent R1	1000 µL
Sample	10 µL

Mix well and incubate for about 5-10 min at 37°C, then add:

Reagent R2	2000 µL
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Mix well, and after 2 min read A₁.

Repeat readings exactly 5 min later -> A₂.

Determine absorbance/min (ΔA/min), calculating **(SECOND readings – FIRST readings) / 5**

CALCULATION

$$\text{G6PDH (U/10}^{12} \text{ RBC)} = \Delta\text{As/min} \times \frac{4777,8}{N}$$

N = Red Cell Count divided by 10⁶

or

$$\text{G6PDH (U/g Hemoglobin)} = \Delta\text{As/min} \times \frac{4777,8}{\text{Hb (g/dL)}}$$

Hb (g/dL) = Hemoglobin conc. determined for each sample