G-6-PDH

(Quantitative Determination of Glucose-6-phosphate Dehydrogenase

Cat.No.	Package Size		
	R1a	R1b	R2
143 000	20 x for 1.0 mL	1 x 20 mL	1 x 40 mL
143 001	8 x for 5.0 mL	2 x 20 mL	2 x 40 mL

TEST PRINCIPLE

G6PDH is an enzyme related to primary blood disorders, related to the RBC. G6PDH presents several genetic variants.The strong reduction of G6PDH activity in a few genetic variants may cause hemolytic disease and crisis, sometimes with fatal outcome.
The test uses the oxidation of Glucose-6-phosphate (G-6-P) to 6-phosphogluconate (6-PG) and simultaneous reduction of NADP to NADPH. The increase of absorbance of NADPH 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

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

Preparation of Working Reagent R1

Cat.No.143000: Add 1 ml Reagent 1b to one vial of Reagent 1a.
Cat.No.143001: Add 5 ml Reagent 1b to one vial of Reagent 1a.
Mix gently to dissolve the relevant lyophilisate..
STABILITY: 5 days at 2-8°C.

Reagent R2 is ready to use

Close vials immediately after use, avoid any contamination!

PRECAUTIONS

- For *in vitro* diagnostic use only.
- The reagent contains < 0,95 g/L sodium azide.
  Avoid contact with skin and/or mucous membranes !

SAMPLES

- 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 is unstable in Red Cell hemolysate –see procedure "Analyzers" Freezing of blood is <u>not</u> recommended

ASSAY PROCEDURE

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

Pipette into test tubes / cuvettes: Calibrator, Controls, Samples :

	Cal/Con	Sample
Working Reagent R1	1000 µL	1000 µL
Calibrator/Control	10 µL	----
Sample	----	10 µL
Mix well and incubate 10 min at 37°C. Then add		
Reagent 2	2000 µL	2000 µL
Mix well, and exactly after 2 min read A_1 of Calibrator, Controls and Samples.		
Exactly after another 5 min read A_2 of Calibrator, Controls and Samples		

Determine absorbance differences (ΔA) for Calibrator ΔA_{CAL} , Control ΔA_{CON} and Sample ΔA_S

ASSAY PROCEDURE ANALYZERS

Ask for individual procedures for special Analyzers.

Note the use of Lysing Reagent – see Note 5

Programming of the Analyzer should be made basically following the Manual Procedure, and considering the individual characteristics of the used instrument. Dilute Samples with Lysing reagent 1 + 9

GENERAL CALCULATION (U/L)

$$\text{G6PDH (U/L 37°C)} = \text{Cal. Value} \times \frac{\Delta A_S}{\Delta A_{CAL}}$$

To receive **U/g Hb** you have to determine for each sample the **concentration of Total Hemoglobin (Hb) in g/dL,** then continue with

CALCULATION (U/gHb)

$$\text{G6PDH (U/g Hemoglobin)} = \Delta As \times \frac{\text{G6PDH (U/L)}}{\text{Total Hb (g/dL)} \times 10}$$

REFERENCE VALUES (Activity at 37°C) Lit.(4)

U/g Hb	**10.0 – 14.2**

Note 1 : Values for Newborns can be higher
Note 2 : Each laboratory should establish its own reference values!

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www.greiner-diagnostic.com

PERFORMANCE CHARACTERISTICS

Linearity: If G6PDH ac is found > 3000) use half sample volume and multiply result x 2.

Sensitivity: The minimum detectable G6PDH activity is 30 U/L

Within-run Precision (n=20)

	Mean (U/L)	CV %
Sample 1	190	2,1
Sample 2	1370	0,8

Day-to-day Precision (n=20)

	Mean (U/L	CV %
Sample 1	190	1,9
Sample 2	1380	0,8

Correlation: 20 sample were assayed by this procedure using a similar commercially available G6PDH Reagent.
Comparison of the data gave following

Linear regression equation y = 1,0007x + 15
Correlation coefficient r = 0,9997

INTERFERENCES

See Literature references (3) and Note 1 below.

NOTES

1. Pay attention to interfering substances like copper and sulfate which are strong inhibitors, but also other substances do influence the measure of the G6PDH levels

2. Reticulocytes have higher G-6-PDH levels than mature Red Cells; it is not recommended to run the assay after a severe hemolytic crisis, since G-6-PDH may appear falsely elevated**.**

3. If the ΔA/min is higher than 0,060 use half sample volume and multiply the result x 2 .

4. If ΔA/min is very low -> increase the sample volume

5. As additional reagent Greiner offers
G6PDH-Lysing Reagent (4 x 25 ml) Cat.No. 507001

BIBLIOGRAPHY

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SYMBOLS USED

For *in vitro* diagnostic medical use

Batch Code


Use by


Temperature limitation

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