G6PDH

Order Information

Cat. No.	Kit size				
1 7900 99 10 026	R1	1 x 20 mL	+	R2	4 x 5 mL
	R3	1 x 40 mL			
1 7900 99 10 040		2 x 0.5 mL	TruCal G6PDH		
		Calibrator set with 2 different levels			

Intended Use

Diagnostic reagent for quantitative in vitro determination of glucose-6-phosphate dehydrogenase (G6PDH) in whole blood on photometric systems.

Summary

Glucose-6-phosphate dehydrogenase (G6PDH) is a cytosolic enzyme that catalyzes the conversion of glucose-6-phosphate (G-6-P) to 6-phophogluconate in the first step in the pentose phosphate pathway. The pentose phosphate pathway is the major source for the NADPH required for anabolic processes. NADPH is required as hydrogen donor for numerous reductive processes as well as for stability of catalase and preservation and regeneration of the reduced form of glutathione. Both, catalase and glutathione are crucial for cell detoxification and cell protection from oxidative stress. Since red blood cells lack any other source of NADPH and are solely dependent on G6PDH, the primary enzyme of the pentose phosphate pathway.
Glucose-6-phosphate dehydrogenase (G6PDH) deficiency is one of the most common human genetic enzymopathies. People with G6PDH deficiency are at risk of hemolytic anemia in states of oxidative stress, infections and after ingestion of certain drugs or fava beans. [1]

Method

Enzymatic UV (photometric) method

Principle

Glucose-6-phosphate dehydrogenase (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 is proportional to the G6PDH concentration in the sample.

The reagent contains 6-PGDH (6-phosphogluconat-dehydrogenase) inhibitors which prevent the production of a second molar equivalent of NADPH by erythrocyte 6-phosphogluconate dehydrogenase.

Reagents

Components and Concentrations

R1:	Good's buffer modified	pH 7.65	> 20 mmol/L
R2:	NADP		> 0.19 mmol/L
R3:	G-6-P	pH 7.65	> 0.1 g/L

Storage Instruction and Reagent Stability

The reagents are stable up to the end of the indicated month of expiry, if stored at 2 – 8°C and contamination is avoided. Do not freeze the reagents.

Warnings and Precautions

1. In very rare cases, samples of patients with gammopathy might give falsified results. [2]
2. Reticulocytes have higher G6PDH levels than mature red cells; it is not recommended to run the assay after a severe hemolytic crisis, since G6PDH may appear falsely elevated.
3. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
4. For professional use only.

Waste Management

Refer to local legal requirements.

Reagent Preparation

Dissolve a vial of R2 with 5 mL of R1 reagent (working reagent), mix gently, and avoid foaming.
Stability: 5 days at 2 – 8°C

R3 is ready to use.

Allow the reagent to reach room temperature before use.
Close immediately after handling.

Materials Required

General laboratory equipment

Specimen

Whole blood collected with EDTA, heparin or ACD (acid-citrate-dextrose).

Sample collection in compliance with CLSI (NCCLS) [3]

Sample Preparation

For sample preparation, DiaSys G6PDH Hemolyzing Solution Cat. No. 1 7900 99 10 113 is required.

Sample preparation:

Hemolyzing Solution	9 parts
Sample/Calibrator/Control	1 part

Mix gently, avoid foaming and assay immediately.

Attention

G6PDH activity is reported in units per gram hemoglobin [U/g Hb] therefore the hemoglobin concentration must be determined prior to performing the G6PDH assay.

Stability

Red cell G6PDH is stable in whole blood for 1 week at 2 – 8°C, but is unstable in red cell hemolysate. A precipitate may appear 20/30 minutes after dilution (see sample preparation with G6PDH Hemolyzing Solution), probably due to the biological variability of the patient's sample. Freezing of blood is not recommended. [4,5]

Assay Procedure

Applications for automated analyzers are available on request.

Wavelength	340 nm (334 – 365 nm)
Optical path	1 cm
Temperature	37°C
Measurement	Against air or distilled water

	Calibrator		Sample
	Level 1	**Level 2**	
Working reagent	1000 µL	1000 µL	1000 µL
Calibrator	10 µL	10 µL	-
Sample	-	-	10 µL
Mix gently and incubate for 10 min. at 37°C, then add:			
R3	2000 µL	2000 µL	2000 µL
Mix gently, read absorbance (A1) after exactly 2 minutes, read absorbance (A2) again after 5 minutes.			

Calculation

Manual calculation of G6PDH activity (U/L – 37°C)

ΔA Calibrator level 1 = A2 calibrator level 1 – A1 calibrator level 1
ΔA Calibrator level 2 = A2 calibrator level 2 – A1 calibrator level 2
ΔA Sample = A2 sample – A1 sample

Calculate ΔA/minute (ΔA/min): ΔA/min = (A2 – A1) / 5

G6PDH (U/L, 37°C) =

ΔA/min x (Total volume/Sample volumen) x (1/ε d) x 1000

Total volume = (1 + 0.01 + 2) = 3.01 mL

Sample volume = 0.01 mL

ε = 6.3 = millimolar absorption of NADPH at 340 nm

d = 1 cm = optical path length

1000 = Factor to convert activity to Liter

G6PDH (U/L, 37°C)

= ΔA/min x (3.01/0.01) x (1/6.3 x 1) x 1000

= ΔA/min x (301 x 1000) / 6.3

= ΔA/min x (301000) / 6.3

= ΔA/min x 47778

Manual calculation of G6PDH activity (U/g Hemoglobin at 37°C)

Considering the value of total hemoglobin (Total Hb) of each sample [g/dL], apply the subsequent formula:

$$\text{G6PDH [U/g Hb]} = \frac{\text{G6PDH [U/L, 37°C]}}{\text{Total Hb [g/dL] x 10}}$$

Calibrators and Controls

DiaSys TruCal G6PDH calibrator is recommended. TruCal G6PDH calibrator values have been made traceable to a commercially available test. Use DiaSys TruLab G6PDH for internal quality control. Each laboratory should establish corrective action in case of deviations in control recovery.

	Cat. No.	Kit size		
TruLab G6PDH (3 levels)	1 7900 99 10 045	3	x	0.5 mL

Performance Characteristics

Data evaluated on MINDRAY BS300

Exemplary data mentioned may slightly differ in case of deviating measurement conditions.

Measuring range up to 3200 U/L When values exceed this range, use half sample volume and multiply the result by 2.	
Limit of detection*	29 U/L

* lowest measurable concentration which can be distinguished from zero;mean + 3 SD (n = 20) of an analyte free specimen.

Interfering substance	**Interferences < 10% up to**
Copper	Strong inhibitor
Sulphate	Strong inhibitor
Ascorbic acid	50 mg/dL
Bilirubin (total)	40 mg/dL
Lipemia (Intralipid®) (triglycerides)	4000 mg/dL
For further information on interfering substances refer to Young DS. [6]	

Precision		
Within run (n=20)	**Sample 1**	**Sample 2**
Mean [U/L]	191	1374
CV [%]	1,4	0,7
Between day (n=20)	**Sample 1**	**Sample 2**
Mean [U/L]	192	1373
CV [%]	1,7	0,9

Method comparison (n=21)	
Test x	Competitor G6PDH
Test y	DiaSys G6PDH
Slope	0.988
Intercept	–13 U/L
Coefficient of correlation	r = 0.991

Conversion Factor

G6PDH [U/L] x 0.0167 = G6PDH [µkat/L]

Reference Range [7]

Adults: 7.9 – 16.3 U/g Hb

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

Literature

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