G-6-PDH
Multi Purpose (MPR) Liquid Reagent

Glenbio
Diagnostics & Research Laboratory Solutions

KIT SPECIFICATIONS:

Cat. No.	*Quantity*	*Reagent*	*Storage*
GL1104GD	4 x 5 ml 4 x 5 ml 4 x 10 ml	G6PDH R1 G6PDH R2 G6PDH R3	2-8ºC

INTENDED USE:

In Vitro Diagnostic reagent pack for the determination of Glucose-6-Phosphate dehydrogenase (G-6-PDH) in whole blood, serum or plasma on automated and semi-automated analysers .

SUMMARY AND EXPLANATION:

G-6-PDH is an enzyme in the Hexose-Monophosphate Shunt (HMS) Pathway. G-6-PDH catalyses the reaction from Glucose-6-P to Phosphogluconate in red blood cells. G-6-PDH presents several genetic variants. A strong reduction in G-6-PDH activity in a few genetic variants may cause, from mild to severe, haemolytic disease, which can occasionally be fatal.

PRINCIPLE OF THE TEST:

Glucose-6-Phosphate Dehydrogenase (G-6-PDH) catalyses the first step in the pentose phosphate shunts, oxidising Glucose-6-phosphate (G-6-P) to 6-phosphogluconate (6-PG) and reducing NADP to NADPH.
The increase in absorbance, due to the formation of NADPH, is proportional to the activity of the G-6-PDH in the sample.

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

WARNINGS AND PRECAUTIONS:

Components Colour and Appearance:
Reagent 1: Clear, colourless liquid
Reagent 2: Lyophilised pellet
Reagent 3: Clear, colourless liquid

Any significant changes could indicate that the assay might be compromised. Refer to Laboratory's QC program for actions to be taken. In case of serious damage to the bottle and/or cap, resulting in product leakage and/or contamination, do not use the reagent pack and contact your distributor.

Safety precautions:
This product is not hazardous under EU specifications. Material Safety Data Sheet is available upon request.

Handling precautions:
- Take the necessary precautions required for handling all laboratory reagents.
- Do not use components past the expiry date stated on the Bottles.
- Do not Freeze Reagents.
- Do not use components for any purpose other than described in the "Intended Use" section.
- Do not interchange caps among components as contamination may occur and compromise test results.
- Refer to local legal requirements for safe waste disposal.

INSTRUMENTS:

Instrument application procedures are available upon request

COMPONENT COMPOSITION:

Component	*Ingredients*	*Concentration in Tests*
Reagent 1, 2 and 3	Good Buffer	>20 mmol/L
	G-6-P	>0.1 g/l
	NADP	>0.19 mmol/L
	Activators, Stabilisers	

REAGENT PREPARATION AND STABILITY:

Reagent 1 & Reagent 2: Add 1 vial of Reagent 1 to 1 vial of reagent 2 and mix gently to dissolve.
This will be stable for 5 days at 2 – 8 ºC
Reagent 3 is ready to use.

Mono-reagent: To prepare a mono working reagent, add the R1 & R2 mixture to 1 bottle of R3 reagent.
This will be stable for 5 days at 2 – 8 ºC.

If stored and handled properly, unopened component is stable up to the expiry date stated on the label.
The components are stable on board the instrument: for 5 days.

TYPE OF SPECIMEN:

Whole Blood, Serum or plasma.

It is recommended to follow CLSI procedures (or similar standardised conditions) regarding specimen handling. Specimen should be collected in an appropriate sampling container, with proper specimen identification. Serum/Plasma should be separated from cells within 2 hours after collection.
Use fresh non-haemolysed serum or plasma.
Stability: G6PDH is stable in whole blood for 1 week at 2-8ºC

TEST PROCEDURE:

Materials required but not supplied:

Description	*Catalog. No.*	*Description*	*Catalog. No.*
G6PDH Calibrator	GL9631	Photometer	N/A
Enzyme Control Level 1	GQC346	General Laboratory Equipment	N/A
Enzyme Control Level 2	GQC347		
Red Cell Lysing Reagent	GLL001		

Assay procedure: Serum/Plasma/Whole Blood

Wavelength	λ: 340 nm
Temperature	37ºC
Optical Path	1cm light path

If using whole blood
Mix 1 part sample with 9 parts red cell lysing reagent prior to assay

	Blank	Calibrator	Sample
Reagent 1+2	1000	1000	1000
Calibrator/Sample	0	10	10
Mix gently and incubate for 10 min at 37ºC, Add			
Reagent 3	2000	2000	2000
Gently Mix .Incubate for 2 minutes then measure the absorbance A1. After a further 5 minutes measure the absorbance again A2. Calculate the absorbance change per minute A2-A1/5			

Calibration:
Using the recommended calibrator calibrate the assay
- When using a new reagent kit or changing lot number.
- Following preventive maintenance or replacement of a critical part of the analyser.
- When Quality Controls are out of range

Quality Control:
All clinical laboratories should establish an Internal Quality Control program. Verify instrument and reagent performance with recommended controls or similar. The values obtained for Q.C. should fall within manufacturer's acceptable ranges or should be established according to the Laboratory's Q.C. program:
Controls should be assayed:
- Prior to reporting patient results.
- Following any maintenance procedure.
- At intervals established by the Q.C. laboratory programme.

CALCULATION:

G6PDH (U/L 37ºC) = (ΔAs/min. Sample / ΔAs/min Calibrator)x Calibrator Conc.

EXPECTED VALUES:

Serum/Plasma 0 – 0.18 U/l
Whole Blood 221-570 U/l [12] RBC or 7 – 20.5 U/g Hb

Each laboratory should establish its own reference range. G-6-PDH results should always be reviewed with the patient's medical examination and history.

PERFORMANCE CHARACTERISTICS:

Performance results can vary with the instrument used. Data obtained in each individual laboratory may differ from these values.

Linearity:
This assay is linear up to 3000 U/L. For sample with a higher activity than this, dilute 1 + 1 with 0.9% NaCl and multiply the result by 2.

Interfering substances:
Copper and sulphate are strong inhibitors.
Recticulocytes are found to have higher G6PDH levels than mature red cells, After haemolytic activity, G6PDH levels may appear falsely elevated

Sensitivity:
The Lowest Detectable Level of G-6-PDH was estimated at 0.8U/g Hb

Precision:

Within Run	Mean *(U/l)*	*SD*	*% CV*	*Between Run*	Mean *(U/l)*	*SD*	*% CV*
Level 1	500	12	2.3	Level 1	526	20	3.8

Method Comparison:
Using 22 samples, a comparison, between this G-6-PDH test (y) and another commercially available test (x), gave the following results:

y = 1.1697x – 89	r = 0.9972

BIBLIOGRAPHY:

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SYMBOLS:

The following symbols are used in the labelling of Glenbio systems:

IVD	In Vitro Diagnostics	REF	Catalogue No
LOT	Batch Code	CONT	Content
REAG	Reagent	Ab	Antibody
CAL	Calibrator	SUBS	Substrate
BUF	Buffer	STD	Aqueous Standard
CE	CE Mark - Device complies with the Directives 98/79/EC		
	Storage temperature	→	Reconstitute with
	Expiry Date (Last day of the month)		Manufactured By
	Biological risk		Consult Instruction for Use

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