INTENDED USE

Glucose-6-Phosphate Dehydrogenase (G6PD) kit is used for the quantitative determination of glucose-6-phosphate dehydrogenase activity in human blood on photometric systems.

SUMMARY

Glucose-6-Phosphate Dehydrogenase (G6PD, EC 1.1.1.49) is a cytosolic enzyme expressed in all cells and plays an important role in pentose phosphate shunt. This enzyme catalyzes the conversion of D-Glucose-6-Phosphate into 6-Phosphoglucono-D-Lactone. During this process, same amount of number of moles of $NADPH^+$ is created. Also it is the rate limiting enzyme of pentose phosphate pathway. (1)

G6PD deficiency is one of the most common enzyme deficiencies around the world, about 400 million, estimated, people have this condition.(2, 3) Most of G6PD-deficient people are usually asymptomatic. People with this enzyme deficiency are at risk of hemolytic anemia during increased oxidative stress.(4) Increased oxidative stress can be the result of many actions, e.g. use of some drugs (e.g. chloroquine), infections, ingestion of fava beans. When anti-oxidative/oxidative balance is disturbed, erythrocytes get damaged. These cells are phagocyted in the spleen. Hemoglobin is degraded into bilirubin, which causes jaundice at higher concentrations.(5)

PRINCIPLE OF ASSAY

The Glucose-6-Phosphate Dehydrogenase Assay kit provides a simple and direct procedure for measuring G6PD activity in whole blood samples. The enzyme activity can be calculated by change of absorbance of $NADP^+$ at 340 nm. The reaction is described below:

G-6-P + $NADP^+$ ➔ 6-PG(6-phosphoglucono-d-lactone) + $NADPH^+ + H^+$

STABILITY AND PREPARATION OF REAGENTS

Reagents:

Contents are ready to use, no preparation is required. Stable up to the expiry date when stored at 2-8˚C after opened.

Controls:

Contents are ready to use, no preparation is required. Stable up to the expiry date when stored at 2-8˚C after opened.

REAGENT COMPOSITION

R1:	Tris Buffer
	NADP >1 mMol
	$MgSO_4$ >10 mMol
	Maleimide >10 mMol
	Preservative Solution (Contains Sodium Azide)
R2:	Tris Buffer
	Glucose-6-Phosphate >5 mMol
	Preservative Solution (Contains Sodium Azide)

CALIBRATION

The assay can be calibrated with deionized water. Calibration in every 30 days is recommended. Calibration is needed whenever the lot number changes. Verify calibration with at least two levels of control according to quality control requirements for your laboratory.

The procedure is standardized by means of the molar absorptivity of $NADPH^+$ taken as $6.22 \times 10^3\ M^{-1}\ cm^{-1}$ at 340 nm under the test conditions described (Optical path: 1cm)

G6PD(U/dL): ΔAbs/min. x 3644(Factor Value) (If optical path is 1 cm. If it is different that this value, please divide the factor by optical path value.)

There may be different Factor Values for different autoanalyzers. Please contact the technical service department for this issue.

One international unit is defined as the amount of the enzyme that catalyzes the conversion of one micromole of substrate per minute under the specified conditions of the assay method.

QUALITY CONTROL

Two levels of controls should be tested every 24 hours.

Control 1: 50 U/dL G6PD (20-80)

Control 2: 200 U/dL G6PD (150-250)

If more intensive control monitoring is required, refer to the quality control procedures developed for your laboratory.

APPLICATIONS ON DIFFERENT AUTOANALYZERS

Application procedures are available for various automated instruments for this G6PD assay. For more information, please contact the Rel Assay Technical Service Department.

FORMULA TO HOW TO CALCULATE G6PD ACTIVITY

The result from autoanalyzer(U/dL) / whole blood sample's hemoglobin concentration(g/dL) = G6PD activity (U/g Hb)

Example:

A whole blood sample's G6PD kit result = 240 U/dL

Whole blood sample Hb concentration = 12 g/dL

G6PD activity of that sample = 240 / 12 = 20 U/g Hb

COMPONENTS

All reagents are ready to use

	Content		Amount
Reagent 1			50 mL
Reagent 2			10 mL
Control 1	50 U/dL G6PD	(20-80)	500 µL
Control 2	200 U/dL G6PD	(150-250)	500 µL

PRINCIPLE OF THE KIT

1. This kit is designed to work on manual spectrophotometer or autoanalyzer.

2. It can be used on different autoanalyzers, an example of application is given below;

Whole blood application

Reaction mode	Rate Up
Wavelength	340/380
Optical path	1cm
Method	Kinetic method

	Volume
Reagent 1	120 µL
Reagent 2	23 µL
Sample	1.5 µL

3. Mix R1, deionized water and sample using pipette. After 5 minutes add R2 to the mixing. After 3 minutes of incubation, take the kinetic measurement.

For the most accurate and sensitive results, make sure that the cuvettes of the spectrophotometer or autoanalyzer used are clean and that the samples are well homogenized.

PERFORMANCE CHARACTERISTICS

Linearity: G6PD kit is linear up to 1350 U/dL.

If the result is greater than upper linearity limit, please dilute the sample with deionized water and multiply the result by dilution factor.

PRECISION

Intra-assay and inter-assay CV were determined with 30 replicates of 20 samples. The intra-assay %CV result 2.5% and inter-assay %CV result was 2.0%.

REFERENCE RANGE

For adults and children;

0 – 7 U/g Hb LOW ACTIVITY

7 - 21 U/g Hb NORMAL ACTIVITY

Above 21 U/g Hb HIGH ACTIVITY

No clinical pathology has been reported for individuals with high activity.

In newborns; results may be physiologically high due to high erythrocyte count.

It is recommended that each laboratory calculates its own reference range.

STORAGE AND VALIDITY DATE

Storage: This kit should be stored at 2-8°C.

Validity date: The validity of reagent is indicated on the bottle and on the kit box.

SAMPLE

Whole blood samples collected with EDTA or heparin are required. Whole blood samples are stable at +4°C for up to 1 week.

In order to express G6PD activity as U/g Hb, hemoglobin values (in g/dL) must also be measured from the samples.

WHOLE BLOOD SAMPLES SHOULD BE MIXED WELL BEFORE TESTING AND MUST BE MEASURED RAPIDLY!

Since the kit measures G6PD activity in erythrocytes, low enzyme results may be encountered on samples that are not mixed well

INTERFERENCES

No interferences were observed with bilirubin up to 40 mg/dL, lipemia up to 4000 mg/dL and ascorbic acid up to 50 mg/dL.

To obtain best results, pipettes and cuvettes used for the assay must be cleaned thoroughly. Make sure that no wash solution is left on pipettes and in cuvettes.

RECOVERY

Whole blood pool was prepared and G6PD activity was measured. Then enzyme activity was re-measured following addition of 1300 U/dL G6PD enzyme solution in ratios of 1/10 and 1/100 into the same pool. As a result, recovery was found in between 90% and 110% range.

SAFETY PRECAUTIONS AND WARNING

1. For IVD uses only.
2. Do not use the kit after its expiry date.
3. Please handle the reagents with caution, avoid ingestion and contact with eyes, mucous membranes and skin.
4. Do not mix kits with different lot numbers.
5. Do not pipette by mouth.
6. The reagents contain sodium azide, do not ingest.
7. The reagents must be used only for the purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.
8. Dispose cleaning liquid and also such used washing cloth or tissue paper with care, as they may also contain infectious agents.

References

1. Rifai N, Horvath AR, Wittwer C. Tietz textbook of clinical chemistry and molecular diagnostics2018.
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3. Nkhoma ET, Poole C, Vannappagari V, Hall SA, Beutler E. The global prevalence of glucose-6-phosphate dehydrogenase deficiency: a systematic review and meta-analysis. Blood Cells, Molecules, and Diseases. 2009;42(3):267-78.
4. Luzzatto L, Seneca E. G6 PD deficiency: a classic example of pharmacogenetics with on-going clinical implications. British journal of haematology. 2014;164(4):469-80.
5. Cappellini MD, Fiorelli G. Glucose-6-phosphate dehydrogenase deficiency. The lancet.2008;371(9606): 64-74.