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G6PDH with BUFFER

Diagnostic reagent for the in-vitro quantitative determination of G6PDH activity in human RBC's using Kinetic method.

REF: BS.1/GP01.005.0005	5 tests	REF: BS.1/GP02.005.0010	10 tests
REF: BS.1/GP05.005.0025	20 tests		

CLINICAL SIGNIFICANCE [1]

Glucose-6-Phosphate-Dehydrogenase (G6PDH) deficiency is one of the most common human enzyme deficiencies in the world. During G6PD deficiency, the red cells are unable to regenerate reduced Nicotineamide adenine dinucleotide phosphate (NADPH), a reaction that is normally catalyzed by the G6PD enzyme. Since the X chromosome carries the gene for G6PD enzyme, this deficiency mostly affects the males. The two major conditions associated with G6PD deficiency are hemolytic anemias and neonatal jaundice, which may result in neurological complications and death. Screening and detection of G6PD deficiency helps in reducing such episodes, through appropriate selection of treatment, patient counselling and abstinence from disease precipitating drugs such as anti-malarial and other agents.

METHOD PRINCIPLE [2]

G-6PDH in the RBC's is released by a lysing agent present in the reagent. The G6PDH released catalyzes the oxidation of Glucose 6 phosphate with the reduction of NADP to NADPH. The rate of reduction of NADP to NADPH is measured as an increase in absorbance which is proportional to the G6PDH activity in the sample.

$$\text{G-6-P} + \text{NADP}^+ \xrightarrow{\text{G-6-PDH}} \text{Gluconate-6–P} + \text{NADPH} + \text{H}^+$$

REAGENT COMPOSITION

R1: Buffer	
Good Buffer modified	> 20 mmol/L
R2: NADP	
NADP	> 0.19 mmol/L
R3: G6P	
G-6-P	> 0.1 g/L
NaN_3	< 0.1 %

PRECAUTIONS AND WARNINGS

Reagent to be handled by entitled and professionally educated person. Do not ingest or inhale as reagent (R2) contains sodium azide which is classified as dangerous substance for environment.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.

S56: dispose of this material and its container at hazardous or special waste collection point.

S57: use appropriate container to avoid environmental contamination.

S61: avoid release in environment.

For further information, refer to the G6PDH reagent material safety data sheet.

REAGENT PREPARATION, STORAGE AND STABILITY

BioScien G6PDH working reagent is prepared by dissolving the content of the vial of R2 with 1mL of R1. Working solution is stable for 6 hours at room temperature and 5 days when stored at 2-8°C. Mix gently avoid foaming. R3 is ready to use. Let the reagent reach the room temperature before use. Close immediately after handling.
All reagents are stable until expiration date stated on label when properly stored refrigerated at 2-8°C.

Deterioration
The ***BioScien*** G6PDH reagent is normally clear, do not use reagent if it is turbid.

SPECIMEN COLLECTION AND PRESERVATION [3]

Blood
Whole blood sample collected in EDTA, Heparin or ACD. Red Cell G-6PDH in whole blood is stable for 7 days at 2-8 °C (Freezing is not recommended) .and unstable in hemolysates. Since activity is reported in terms of number of red cells or grams of hemoglobin. The red cell count or hemoglobin concentration should be determined prior to performing the G-6PDH assay. The integrity of erythrocytes collected in ACD is preserved even after prolonged storage so that obtaining accurate red cell counts usually poses no problem. However, red cell counts on specimens collected in heparin become unreliable after about 2 days. Thus, for heparinized samples, results are best reported in terms of hemoglobin concentration.

SYSTEM PARAMETERS

Wavelength	340 nm (334 – 365)
Optical path	1 cm
Assay type	Kinetic
Direction	Increase
Sample Reagent Ratio egg: Reagent volume Sample volume	1:300 3 ml 10 µl
Temperature	37˚C
Incubation time	5 min.
Zero adjustment	Air or Distilled Water
Factor	48390

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile Syringe
- Analytical tubes, automatic pipet

ASSAY PROCEDURE

	Specimen
Working Reagent	250 µl
Specimen	5 µl
Mix kindly and incubate for 10 minutes at *37°C*.	
R 3	0.5 ml

Mix well and incubate for 2 min. Read the initial absorbance, repeat absorbance reading after 5 minutes. Calculate the mean absorbance change per minute (Δ A / min.)
For concentration of G6PDH higher than 3200 U/L use half sample volume and multiply the result x 2.

CALCULATION [3]

G6PDH activity is expressed as U/L or U/g hemoglobin (Hb).
G6PDH (U/L) = Δ A/min x 48390

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

Where '10' is the multiplier that converts g/dl in g/l of total hemoglobin (Total Hb)

$$\text{G6PDH (U/10}^{12}\text{ RBC's)} = \frac{\text{G6PDH (U/L)}}{\text{RBC count in million}}$$

Use of Buffy-Coat-Free Sample [4,5]

Under normal circumstances G6PD activity contributed by leukocytes, platelets and serum is relatively small. However, as reported by Echler and others, more accurate measurement of red cell G6PD activity, especially in the presence of anemia and/or leukocytosis, can be achieved by using buffy coat-free blood samples for assay. Thus, in case of a borderline value obtained with whole blood, it may be warranted to repeat the assay on a buffy coat-free sample.

QUALITY CONTROL

To ensure adequate quality control, it is recommended that normal and abnormal commercial control of known concentrations included in each run. If control values are found outside the defined range, check the instrument calibration, and reagent for problems. If control still out of range please contact ***BioScien*** technical support.

PERFORMANCE CHARACTERISTICS

Precision	Within run (Repeatability)		Run to run (Reproducibility)	
	Normal level	High level	Normal level	High level
n	20	20	20	20
Mean IU/L	264	537	276	535
SD.	13.6	23.7	16.0	25.5
CV. %	5.2	4.4	5.8	4.8

The results of the performance characteristics depend on the analyzer used.

Accuracy (Methods Comparison)

Result obtained from ***BioScien*** G6PDH reagent compared with commercial reagent of the same methodology give a correlation of 0.990.

Sensitivity

When run as recommended, the minimum detection limit of the assay is 27 U/L.

Linearity

The Assay is linear up to G6PDH concentration of 3200 U/L.

INTERFERING SUBSTANCES [6,7]

Icterus

Bilirubin has negative interference above 7.5 mg/dL.

lipemia

Lipemic specimens interfere up to 4000 mg/dl.

Ascorbic acid

It interfere up to 50 mg/dl.

Others

Glucose, turbidity, copper and other drugs may interfere.

EXPECTED VALUES [6]

7.9 – 16.3 U/g Hb

202 – 522 U/10^{12} RBC's

Values for the newborns may range somewhat higher.
Since the normal values depend on age, sex, diet, geographic area and other factors, each laboratory should establish its own normal values for this procedure.

REFERENCES

1. Tietz N. W., textbook of clinical chemistry. Burtis CA, Ashwood ER, Saunders W.B. 3rd Edition, (1999) p 1645-1250.
2. Beutler, E., Blume, K.G., Kaplan, C., Lohr, W., Ramont, B., Valentine, W.N.: International committee for standardization in hematology: "Recommended Methods for Red Cell Enzyme Analysis". Bri J of Haem, 35, p,331-340, 1977.
3. WHO Technical Report Series No. 366, Standardization of Procedures for the study of Glucose-6-Phosphate Dehydrogenase, 1967.
4. Echler, G.: Determination of glucose-6-phosphate dehydrogenase. Am J Med Technol 49:259, 1983.
5. Morelli, A., Benatti, U., Lenzerini, L., Sparatore, B. et al: The interference of leukocytes and platelets with measurement of glucose-6-phosphate dehydrogenase activity of erythrocytes with low activity variants of the enzyme. Blood 58: 642, 1981.
6. Tietz N. W., Clinical Guide to Laboratory Tests, 4th Edition, (2006) p 457-458.
7. Young D.S., Effects of drugs on clinical laboratory tests. 4th Ed. (1995), p.3-216 to 3-216.

SYMBOLS IN PRODUCT LABELLING

IVD	For in-vitro diagnostic use	Σ	Number of <n> test in the pack
LOT	Batch Code/Lot number		Caution
REF	Catalogue Number		Do not use if package is damaged
	Temperature Limitation		Consult Instruction for use
	Expiration Date		
	Manufactured by		

EC REP **Medical Device Safety Service**
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CE