DIALAB Produktion und Vertrieb von chemisch – technischen Produkten und Laborinstrumenten Gesellschaft m.b.H.
A – 2351 Wiener Neudorf, Austria, IZ-NÖ Süd, Hondastrasse, Objekt M55
Phone: ++43 (0) 2236 660910-0, Fax: ++43 (0) 2236 660910-30, e-mail: office@dialab.at

Dry Powder Reagents

G6PDH

(Glucose-6-Phosphate Dehydrogenase)

UV, kinetic
2 Reagents

Diagnostic reagent for quantitative in vitro determination of Glucose-6-phosphate dehydrogenase in human blood on photometric systems

REF	Kit Size	Content
Y04500	**10 x 18 mL**	10 x 6 mL R1 + 1 x 120 mL R2

Additionally offered:

Y17565SV	1 x 0.5 mL	G6PDH Calibrator
Y17560	6 x 0.5 mL	G6PDH Control Set

TEST PARAMETERS

Method:	UV, kinetic, increasing reaction
Wavelength:	340 nm
Temperature:	37°C
Sample:	Whole blood with EDTA, heparin or acid-citrate-dextrose (ACD)
Linearity:	Up to 21.0 U/g Hb or 609 U/10^{12} RBC
Sensitivity:	0.4 U/g Hb or 11 U/10^{12} RBC

SUMMARY [1]

G6PDH assays are most commonly performed to determine deficiency of G6PDH, which is widely prevalent throughout the world. It has been determined that G6PDH deficiency in red ells is the basis for certain drug-induced haemolytic anemias. This type of susceptibility to drug-induced hemolysis is often called “primaquine sensitivity” because studies which led to its characterization were made during investigations of the haemolytic properties of this antimalarial compound.

TEST 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:

$$\text{G-6-P} + \text{NADP}^+ + \xrightarrow{\text{G6PDH}} \text{6-PG} + \text{NADPH} + \text{H}^+$$

NADP is reduced by G6PD in the presence of G-6-P. The rate of formation of NADPH is proportional to the G6PDH activity and is measured as increase in absorbance at 340 nm.
Production of a second molar equivalent of NADPH by erythrocyte 6-phosphogluconate dehydrogenase (6-PGDH) is prevented by use of maleimide, an inhibitor of 6-PGDH.

REAGENT COMPOSITION

COMPONENTS	CONCENTRATION
Reagent 1:	
NADP	1.5 mM
Maleimide	12 mM
Buffer, stabilizers, lysing agent	
Reagent 2:	
Glucose-6-phosphate	1.05 mM
Magnesium salt, buffer	

REAGENT PREPARATION

Reagent 1: For procedure on automatic instruments reconstitute the content of each vial with the volume of deionized water indicated on the label. Swirl gently and invert several times to dissolve contents. Wait 2-3 min. and mix again.
For manual test procedure add Lyse Reagent as the diluent instead of deionized water!
Reagent 2: Reagent 2 is ready to use.

REAGENT STABILITY AND STORAGE

Conditions:	protect from light, close immediately after use Do not freeze the reagents!	
Storage:	at 2 – 8°C	
Stability:	up to the expiration date	
After reconstitution:		
Reagent 1:	at 18 – 26 °C	8 hours
	at 2 – 8 °C	5 days

SAMPLE COLLECTION AND PREPARATION

Whole blood collected in EDTA, heparin or acid-citrate-dextrose (ACD) is satisfactory [4-8].
Since activity is reported in terms of grams haemoglobin or the number of red blood cells, the haemoglobin concentration or red cell count must be determined prior to performing the G6PDH 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 [6]. However, red cell counts on specimens collected in heparin become unreliable after about 2 days [6]. Thus, for heparinized samples, results are best reported in terms of haemoglobin concentration.
We suggest: Y04701 Hemoglobin Total Reagent.
For performing tests on automatic instruments, add 100 µl whole blood to 0.9 ml G6PDH Lyse Reagent and let stand for 5 minutes. Mix well. Use hemolysate as sample.

SAMPLE STABILITY AND STORAGE [9]

Whole blood:	at 2 – 8 °C	7 days
Hemolysate:	unstable	

Do not freeze! Discard contaminated specimens.

MATERIALS REQUIRED BUT NOT PROVIDED

General laboratory equipment
Equipment and reagents for determining haemoglobin concentration or performing a red cell count.

MANUAL TEST PROCEDURE

Reconstitute R1 by adding Lyse Reagent as the diluent instead of deionized water.

Pipette into a cuvette:	
Reagent 1	1000 µL
Sample / Controls	10 µL
Mix thoroughly to completely suspend erythrocytes. Let stand at room temp. (18 – 26 °C) for 5 – 10 min. Then add R2:	
Reagent 2	2000 µL
Mix gently by inverting several times. Incubate at 37 °C for 5 minutes and read absorbance A1 at 340 nm vs. water. Incubate again for exact 5 min at 37°C and read absorb. A2.	

CALCULATION (light path 1 cm)

$$\Delta A/\text{min} = \frac{A2 - A1}{5}$$

G6PDH activity can be expressed as either U/g hemoglobin (Hb) or as U/10^{12} erythrocytes (RBC):

$$\text{G6PDH [U/g Hb]} = \Delta A/\text{min} \times \frac{100 \times 3.01}{0.01 \times 6.22 \times \text{Hb (g/dL)}} \times \text{TCF}$$

$$= \Delta A/\text{min} \times \frac{4839}{\text{Hb (g/dL)}} \times \text{TCF}$$

$$\text{Or: G6PDH (U/}10^{12}\text{ RBC)} = \frac{\Delta A/\text{min} \times 3.01 \times 10^{12} \times \text{TCF.}}{0.01 \times 6.22 \times (\text{N} \times 10^6) \times 1000}$$

$$= \Delta A/\text{min} \times \frac{48390}{\text{N}} \times \text{TCF}$$

100 = Factor to convert activity to 100 mL
3.01 = Total reaction volume (mL)
0.01 = Sample volume (mL)
6.22 = millimolar absorptivity of NADPH at 340 nm
Hb (g/dL) = Hemoglobin concentration for each specimen
TCF = Temperature Correction Factor (= 1 at 37 °C)
10^{12} = Factor for expressing activity in 10^{12} cells
(Nx10^6) = Red cell count (red cells/mm^3) for each specimen
N = red cell count divided by 10^6
1000 = conversion of red cell count from mm^3 to mL

TEMPERATURE CORRECTION [14]

Cuvette temperature	TCF
25 °C	1.98
30 °C	1.37

REFERENCE RANGE [1] *

The following ranges were obtained by examinating 98 clinically healthy males and females:

at 37°C	290 - 412 U / 10^{12} RBC	10.0 – 14.2 U / g Hb

Values for newborns may range somewhat higher.

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

Rev. 05, 08.01.2018

DIALAB Produktion und Vertrieb von chemisch – technischen Produkten und Laborinstrumenten Gesellschaft m.b.H.
A – 2351 Wiener Neudorf, Austria, IZ-NÖ Süd, Hondastrasse, Objekt M55
Phone: ++43 (0) 2236 660910-0, Fax: ++43 (0) 2236 660910-30, e-mail: office@dialab.at

PERFORMANCE CHARACTERISTICS

LINEARITY

The maximum G6PDH activity which may be measured by this procedure is appr. 21.0 U/g Hb or 609 U/10^{12} RBC.
If ΔA/min is greater than 0.06, repeat determination using 5 µL blood as sample and multiply results by 2.

SENSITIVITY

Minimum change in absorbance at 340 nm: ΔA/min = 0.001.
Assuming a hemoglobin concentration of 12.0 g/dL and a red cell count of 4.5x10^6/mm^3, a G6PDH activity of 0.4 U/g Hb or 11 U/10^{12} RBC may be detected.

PRECISION

Intra-assay n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	257	23.7	9.2
Sample 2	658	18.3	2.8
Sample 3	1939	48.0	2.5

Inter-assay n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	269	30.8	11.4
Sample 2	700	28.7	4.1
Sample 3	2014	43.0	2.1

SPECIFICITY/INTERFERENCES

The oxidation of glucose-6-phosphate by G6PDH is specific. Any non-specific formation of NADPH due to oxidation of other substrates by endogenous enzymes occurs during the preincubation period.
6-Phosphogluonate dehydrogenase is completely inhibited by maleimide in the reagent system.

- Copper completely inhibits G6PDH at a concentration of 100 µmol/L, and sulfate ions (0.005 mol/L) decrease observed levels of G6PDH activity [10].
- Certain drugs and other substances are known to influence circulation levels of G6PDH [11].
- Reticulocytes have higher G6PDH levels than mature red cells. It is recommended that assays not be performed after a severe haemolytic crisis, since G6PDH levels may appear falsely elevated. Under those conditions, detection of deficiency may require family studies. Testing may be performed after the level of mature red cells has to returned to normal.
- Under normal circumstances, activity contributed by leukocytes, platelets and serum is relatively small. However, as reported by Echler [12] and others [13], more accurate measurement of red cell G6PDH activity, especially in the presence of anemia, and/or leucocytosis, 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.

METHOD COMPARISON

A comparison study between Dialab G6PDH (y) and a commercially available test (x) gave the following results:
y = 0.97 x + 0.07; r = 0.994

CALIBRATION

The procedure is standardized on the basis of the millimolar absorptivity of NADPH, which is 6.22 at 340 nm. Measurement of the rate of increase in absorbance (ΔA) at 340 nm serves to quantitate enzymatic activity.
The use of a calibrator is optional. We recommend the Dialab **G6PDH Calibrator**.

QUALITY CONTROL

Reliability of test results should be monitored by use of control materials with known values within each run. We recommend the Dialab **G6PDH Control Set**.
Each laboratory should establish corrective action in case of deviations in control recovery.

AUTOMATION

Special applications for automated analyzers can be made on request.

WARNINGS AND PRECAUTIONS

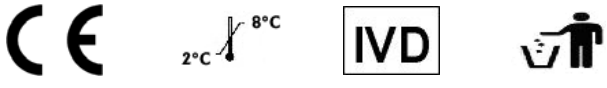
1. Reagent 1: Danger. H302: Harmful if swallowed. H314: Causes severe skin burns and eye damage. H317: May cause an allergic skin reaction. H318: Causes serious eye damage. H335: May cause respiratory irritation. P260: Do not breathe dust/fume/gas/mist/vapours/spray. P264: Wash hands and face thoroughly after handling. P270: Do not eat, drink or smoke when using this product. P271: Use only in a well-ventilated area. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves/protective clothing/eye protection. P301+P330+P331: IF SWALLOWED: Rinse mouth. Do NOT induce vomiting. P302+P352: IF ON SKIN: wash with plenty of soap and water. P303+P361+P353: IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse SKIN with water/shower. P304+P340: IF INHALED: Remove person to fresh air and keep comfortable for breathing. P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P310: Immediately call a POISON CENTER or doctor/physician. P312: Call a POISON CENTER or doctor/physician if you feel unwell. P333+P313:If SKIN irritation or rash occurs: Get medical advice/attention. P363: Wash contaminated clothing before reuse. P403+P233: Store in a well-ventilated place. Keep container tightly closed. P501: Dispose of contents into sewer system after diluting with large volumes of water, if in accordance with local regulations.
2. Reagent 2 contains sodium azide (< 0.1 g/L) which may react with lead and copper plumbing to form highly explosive metal azides. Avoid azide accumulation.
3. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents.
4. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
5. For professional use only!

WASTE MANAGEMENT

Please refer to local requirements.

REFERENCES

1. Burtis, C.A., Ashwood, E.R., Tietz Textbook of Clinical Chemistry, W.B. Saunders, Philadelphia, pp. 1645-1650, 1999.
2. Kornberg, A., Horecker, B.L.: Glucose-6-Phosphate Dehydrogenase. IN Methods in Enzymology. S.P. Colowick, N.O. Kaplan, Editors, Vol. I, Academic Press, New York, p 323, 1955.
3. Lohr, G.W., Waller, H.D.: Glucose-6-phosphate Dehydrogenase. IN Methods of Enzymatic Analysis. H.U. Bergmeyer, Editor, Academic press, New York, p. 636, 1974.
4. Kachmar, J.F., Moss, D.W.: Enzymes. IN Fundamentals of Clinical Chemistry. N.W. Tietz, Editor, Saunders, Philadelphia, pp. 66-672, 1976
5. WHO Technical Report Series No. 336, Standardization of Procedures fort he study of Glucose-6-Phosphate Dehydrogenase, 1967.
6. Lowe, M.L., Stella, A.F., Mosher, B.S., Gin, J.B., Demetriou, J.A.: Microflourometry of Glucose-6-phosphate dehydrogenase and 6-phosphoglucanate Dehydrogenase in red cells. Clin Chem 18:440, 1972.
7. Bishop, C.: Assay of glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and Glucose-6-phosphate dehydrogenase (EC 1.1.1.44) in red cells. J Lab Clin Med 68: 149, 1966.
8. Beutler, E., Blume, K.G., Kaplan, C., Lohr, W., Ramont, B., Valentine, W.N.: International committee for standardization in haematology: Recommendedscreening test for glucose-6-phosphate dehydrogenase (G6PD). Bri J of Haem, 43:469-477, 1979.
9. Stiene, E.A.: Red Cell Enzyme Deficiencies: A review: Am J Med Tech 38:454, 1972.
10. Boulard M, Blume KG, Beutler E. The effects of copper on red cell enzyme activities. J. Clin Invest, 51, 459 (1972).
11. Young, D.S., Pestaner, L.C.., Gibberman, V.: Effects of drugs on clinical laboratory tests. Clin Chem 21: 302D, 1975.
12. Echler, G.: Determination of glucose-6-phosphate dehydrogenase. Am J Med Technol 49:259, 1983.
13. 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.
14. Beutler, E., et al, International Committee for Standardization in Haematology: Recommended methods for red-cell enzyme analysis. Br. J. Haematol., 35:331-340, 1977
15. NCCLS document "Evaluation of Precision Performance of Clinical Chemistry Devices", 2nd Edition, 1992.



Rev. 05, 08.01.2018