EXPECTED VALUES

G6PDH Activity (at 30°C) : 146 - 376 U/10^{12}RBCs

: 4.6 - 13.5 U/g Hb

(at 37°C) : 203 - 523 U/10^{12} RBCs

: 6.4 - 18.8 U/g Hb

NOTES

If the G6PDH activity is very low, measure the absorbance change for 5 minutes after addition of buffered substrate and divide by 5 to obtain Δ A/min. and calculate the test results.

QUALITY CONTROL

To ensure adequate quality control, it is recommended that each batch should include normal and abnormal reference control. It should be realised that the use of quality control material checks both instrument and reagent functions together. Factors which might affect the performance of this test include proper instrument function, temperature control, cleanliness of glassware and accuracy of pipetting.

REFERENCES

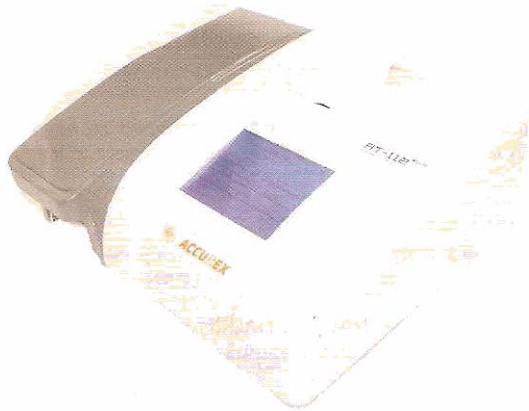
1. Gross, R.T., Hurwitz R. E., Marks P. A.: Red Cell Glucose -6- Phosphate Dehydrogenase deficiency. ***J. Clin. Invest (1978) 37.176.***
2. Kachmar, J. F., Moss, D. W., : Enzymes. In Fundamentals of ***Clinical Chemistry*** Ed. by N. W. Tietz, Saunders Philadelphia (1976) pp 666-672.

	In Vitro Diagnostic Use		Date of Manufacturing
	Consult Instructions for use		Use by (YYYY-MM-DD)
REF	Catalogue Number		Temperature Limitation
	Batch Code		Manufacturer

NEW

AT-112 PLUS

Biochemistry Analyzer



Semi Automated Clinical Chemistry Analyzer

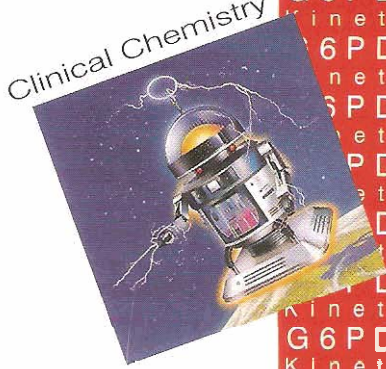
AR. No : I 100 *GP2-2011-11-001*

ACCUREX BIOMEDICAL PVT. LTD.

Head Office - Mumbai. Tel.: 91(022) 67446744; Fax: 91(022) 67446755

E-mail: accurex@vsnl.com; Website: www.accurex.org

Plant : G-54, MIDC Tarapur, Boisar, Thane - 401 506. INDIA.



AutoZyme

NEW

G6PDH

Quantitative - Kinetic

G6PDH • G6PDH
Kinetic • Kinetic

INTRODUCTION

1. AutoZyme NEW G6PDH is a reagent set for **quantitative determination of G6PDH activity** in erythrocytes by kinetic method.
3. AutoZyme NEW G6PDH can be used on any **semi-automated analyzer.**

PRINCIPLE

G6PDH present in the erythrocytes (RBCs) is extracted by lysing the cells using lysing reagent. The extracted enzyme catalyzes the following reaction :

$$\text{Glucose-6-Phosphate} + \text{NADP} \xrightarrow{\text{G6PDH}} \text{6-Phosphogluconate} + \text{NADPH}$$

The conversion of co-enzyme NADP to NADPH is proportional to the concentration of G6PDH activity in the sample and is measured at 340 nm as rate of increase in absorbance.

PREPARATION OF WORKING SOLUTION

Reconstitute one vial of R1 with 1.1 ml of R2 to prepare working solution. Mix by gentle swirling or inversion. Do not shake vigorously.

G6PDH R3 is ready-to-use and should be used pre-cooled at 2 - 8°C.

REAGENT STORAGE & STABILITY

The working solution is stable for 7 days at 2 - 8°C.

The reagent kit should be stored at 2 - 8°C and is stable till the expiry date indicated on the label.

REAGENT COMPONENTS

	12 ml
R1 : Co-enzyme/Substrate	: 12 vials
R2 : Buffer	: 1 bottle
R3 : Lysing Reagent	: 1 bottle

SPECIMEN COLLECTION & PRESERVATION

Whole blood should be collected in a clean and dry container with citrate or oxalate.

Determine the Haemoglobin content of the whole blood or the RBC count prior to lysis of the cells.

PROCEDURE

- ☐ **Reaction type**.....**Kinetic**
- ☐ **Reaction direction** .....**Increasing**
- ☐ **Wavelength** .....**340 nm**
- ☐ **Flowcell temp**.....**30°C/37°C**
- ☐ **Zero setting with** .....**Distilled water**
- ☐ **Delay time**.....**180 seconds**
- ☐ **No. of readings**.....**4**
- ☐ **Interval**.....**60 seconds**
- ☐ **Sample volume** .....**0.025 ml (25 µl)**
- ☐ **Working solution volume** .....**1.0 ml (1000 µl)**

STEP I : PREPARATION OF RED CELL HAEMOLYSATE

Wash 0.1 ml of whole blood with 2 ml of physiological saline (0.9% NaCl) 3 times and suspend the washed, packed and centrifuged erythrocytes in pre-cooled 0.5 ml of G6PDH R3 (Lysing Reagent). Mix well and keep in the refrigerator (2 - 8°C) for at least 15 minutes and maximum for 2 hours. Centrifuge the lysate at 3000 r.p.m. for 5 minutes prior to use as given below:

STEP II : MANUAL ASSAY PROCEDURE

Reagents	Test
Working Solution	1.0 ml
Haemolysate	25 µl

Mix and aspirate. After the initial delay of 180 seconds, record the absorbance of the test at an interval of one minute for the next 3 minutes at 340 nm. Determine the mean change in absorbance per minute and calculate test results.

CALCULATION

1. G6PDH Activity (U/10^{12} RBC)

$$= \frac{\Delta\ A/Min\ \times 224 \times 10^{12}}{6.22 \times N \times 10^{6} \times 1000}$$

$$= \frac{\Delta\ A/Min \times 36013}{N}$$

Where 224 = Total assay volume to sample volume

10^{12} = Factor for expressing activity in 10^{12} cells

6.22 = Millimolar absorptivity of NADPH at 340 nm

N x 10^{6} = No. of erythrocyte/cmm

1000 = Conversion of cell count from count per cmm to count per ml

2. G6PDH Activity (U/g Hb)

$$= \frac{\Delta\ A/Min \times 224 \times 100}{6.22 \times Hb\ (g/dl)}$$

$$= \frac{\Delta\ A/Min \times 3601}{Hb\ (g/dl)}$$

Where 224 = Total assay volume to sample volume

100 = Factor to convert to 100 ml

6.22 = Millimolar absorptivity of NADPH at 340 nm

Hb (g/dl) = Haemoglobin concentration

GP1-2011-11-001