GLUCOSE-6-PHOSPHATE DEHYDROGENASE

ENZOPAK
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(UV-Kinetic Method)
Reagent kit for quantitative estimation of G6PDH in erythrocytes.

1. Perhaps the only company in India making both visual (dye) reagent and UV assay reagents.
2. Reagent ensuring complete inhibition of 6-Phosphogluconate Dehydrogenase, thereby giving accurate results.
3. A very convenient pack size.
4. Q.A. procedures to ensure very high quality, high accuracy.

INTRODUCTION:
Hemolytic anaemias or hemolytic episodes are related in most of the cases to enzyme deficiencies due to hereditary abnormalities. There are many screening nonspecific tests like osmotic fragility autohemolysis tests etc. Additional better screening tests for metabolic defects in red cell are to measure glucose consumption, lactate production or measure contribution of pentose phosphate pathway to metabolism. However, these tests being elaborate and difficult and still not being specific, it is better to identify these deficiences by enzyme assays.
One of the common enzyme deficiencies for hemolytic episodes/ hemolytic anaemia is measurement of Glucose-6-Phosphate Dehydrogenase, by a quantitative enzyme assay.
Enzopak G-6-PD uses a potent inhibitor to prevent interference caused by 6-Phosphogluconate dehydrogenase.

PRINCIPLE:
The enzyme Glucose-6-Phosphate Dehydrogenase present in the Red Blood Cells is extracted by lysing the cells using a natural detergent. The extracted enzyme oxidises Glucose-6-Phosphate to 6-Phosphogluconate and simultaneously reduces co-enzyme NADP to NADPH giving increase in absorbance at 340 nm.

Enzymatic determination of G6PDH activity is based on the following reaction.

The reaction is as shown.

DIAGNOSTIC SIGNIFICANCE:
G-6-PD deficient subjects are prone to hemolytic episodes and hemolytic anaemia if treated with certain antimalarial or sulfadrugs. The screening for the deficiency is one of the first preventive step to avoid these hemolytic episodes and anaemia.
The estimation will indicate deficiency better in males than in females.

REAGENT COMPOSITION:

Active Ingredients	Concentration
Reagent-1	
* Glucose-6-Phosphate	0.5 mmol/L
* NADP Na_2	0.75 mmol/L
Reagent-2	
* Buffer	100 mmol/L
pH 7.5 ± 0.1 at 25^0 C	
Reagent-3	
* Detergent	1 mmol/L

Also contains non-reactive fillers and Stabilizers.

PRESENTATION:

	No. of Bottles
Store all reagents at 2-8^0 C	12 x 1.1 ml.
1. G6PDH (Co-enzyme-substrate)	12
2. G6PDH (Buffer)	1
3. G6PDH (Lysing Reagent)	1

PRECAUTION:
ENZOPAK G6PDH is for *IN-VITRO* diagnostic use only.
Reagent contains Sodium Azide, DO NOT INGEST.
The Lysing Reagent (3G6PDH) should be used cold (0-8 ^{0}C) to avoid the decrease in enzyme activity.

SPECIMEN COLLECTION AND PRECAUTIONS:
Fresh whole blood is the specimen required. Collection of blood by using any one of the anticoagulants such as citrate, Oxalate or Heparin is recommended.
Determine the Hemoglobin content of the whole blood and the RBC count prior to lysis of the cells.

PREPARATION OF RED CELL HEMOLYSATE :
Wash 0.1 ml of whole blood with 2 ml. aliquots of physiological saline (0.9%) 3 times, and suspend the washed, packed and centrifuged erythrocytes in precooled 0.5 ml of 3G6PDH (Lysing reagent). Mix well and keep in the refrigerator (2-4 ^{0}C) for atleast 15 minutes and maximum for 2 hours. Centrifuge the lysate at 3000 r.p.m. for 5 minutes prior to use.

PREPARATION OF REAGENT:
Add 1.1 ml. buffer (2G6PDH) to the Vial labelled 1G6PDH. Mix well and use after five minutes.

REAGENT STORAGE AND STABILITY :
ENZOPAK G6PDH reagents are stable until the expiry date stated on the label. Reconstituted reagent is stable for 1 week at 2-8 ^{0}C.

GENERAL PARAMETERS :

- Type of Reaction : Kinetic / Increasing OD
- Wavelength : 340 nm
- Flowcell Temperature : 30^{0}C
- Delay Time : 180 Seconds
- Interval : 60 Seconds
- No. of reading : 4
- Sample Volume : 25 µl (0.025 ml)
- Working Reagent volume : 1.0 ml
- Zero setting with : Distilled Water
- Light Path : 1.0 cm.

PROCEDURE

PIPETTE INTO TEST TUBES	TEST
• WORKING REAGENT (ml)	1.0
• HEMOLYSATE (ml)	0.025

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

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TEST RESULTS:

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

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

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

Where 224 = Total assay volume to sample volume.
10^{12} = Factor for expressing activity in 10^{12} cells.
6.22 = Milimolar absorptivity of NADPH at 340 nm.
$N \times 10^{6}$ = Number of erythrocytes / cmm.
1000 = Conversion of cell count from count per cmm to count per ml.

(ii) G6PDH Activity (U/g Hb)

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

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

Where 100 = Factor to convert to 100 ml.
224 = Total assay volume to sample volume.
6.22 = Milimolar absorptivity of NADPH at 340 nm.
Hb (g/dl) = Hemoglobin concentration.

EXAMPLE:

Assay of specimen which had a red cell count of 4.6×10^{6} / mm^3 and a hemoglobin concentration of 15.2 g/dl resulted in ΔA/min at 30 ^{0}C of 0.028.

$$G6PDH\ (U/10^{12}\ RBC) = \frac{0.028 \times 36013}{4.6} = 219$$

$$G6PDH\ (U/gHb) = \frac{0.028 \times 3601}{15.2} = 6.83$$

NORMAL VALUES:

G6PDH Activity (at 30^{0}C) 146-376 U/10^{12} RBCs
4.6-13.5 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 ΔA/minute and calculate the test results. Use the formula given under 'TEST RESULTS'.

REFERENCES:

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3/7,B.I.D.C., Gorwa, Vadodara 390 016 (INDIA)
Web: www.reckondiagnostics.com
Phone: +91·265·2281631
Email: mail@reckondiagnostics.com

RECKON DIAGNOSTICS P. LTD.