ESTROM G-6-PD (Lypho)

(UV Kinetic Method)

ANGSTROM BIOTECH PVT. LTD

This reagent kit is for quantity estimation of G6PDH activity in erythrocytes.

PRINCIPLE:

The sustrate Glucose-6-Phosphate is oxidized to 6 phosphogluconate with simultaneous reduction of NADP to NADPH. This reaction is catalysed by Glucose-6-Phosphate dehydrogenase. Increased in absorbance is read at 340nm.

A natural detergent is used to lyse the RBC's for the extraction of Glucose-6-Phosphate dehydrogenous.

$$\text{GLUCOSE-6-PHOSPHATE} + \text{NADP} \xrightarrow{\text{G6PD}} \text{6 PHOSPHOGLUCONIC ACID} + \text{NADPH}$$

CLINICAL SIGNIFICANCE:

G6PHD deficiency is more pronounced in males than in females. Screening the deficiency is the first requisite step to avoid hemolytic episodes & anemia. Treatment with certain anti-malarial or sulfa drugs in case of G6PD deficient subject can lead to hemolytic episodes and hemolytic anemia.

SPECIMEN COLLECTION AND STORAGE:

- Fresh whole blood is necessary.
- Citrate, oxalate, or heparin can be used as anticoagulant.
- Determine Hb content of whole blood or RBC count.

PRECAUTION:

- Esrom reagents are for invitro diagnostic use only

REAGENTS:

All the reagents are to be stored at 2-8^0 C.

	No. of bottles 12x1 ml
Reagent 1 (Coenzyme-substrate)	12
Reagent 2 (Buffer)	1
Reagent 3 (Lysing Reagent)	1

REAGENT RECONSTITUTION:

12 x 1 ml: Dissolve the contents of 1 vial Reagent 1 G6PD using 1.0 ml of Reagent 2 G6PD.

Mix well. Store at 2-8°C when not in use.

REAGENT STORAGE AND STABILITY:

Estrom G6PD reagents are stable until the expiry date stated on the label. Reconstituted reagent is stable for 1 week at 2-8°C

GENERAL INSTRUMENT PARAMETERS:

Reaction type	: Kinetic
Wavelength	: 340 nm
Flowcell Temperature	: 30 °C/37^{0}C
Delay Time	: 30 seconds
Interval	: 60 seconds
No. of Readings	: 4
Sample Volume	: 5 µl
Working Reagent Volume	: 1.5 ml
Zero Setting	: Distilled water
Path length	: 1.0 cm

PROCEDURE:

For 1.5 ml

Dispense into test tubes	Test
Reagent 3	0.5 ml
Whole blood	5 µl
Mix well & incubate for 5-10 minutes at RT, then add	
G6PD working reagent	1 ml

Mix well and incubate at 30^{0}C/37^0 C and read the initial absorbance Ao & repeat the absorbance reading at after every 1, 2 & 3 minutes. Calculate the mean absorbance change per minute (ΔA/min).

CALCULATION:

$$\text{G6PDH Activity (U/10}^{12}\text{RBC)} = \Delta A/\text{min} \times \frac{47780}{\text{RBC count in million}}$$

$$\text{G6PDH Activity (U/g Hb)} = \Delta A/\text{min} \times \frac{4778}{\text{Hb (g/dl)}}$$

NORMAL VALUES:

G6PD Activity (Ug/Hb)	: 4.6 – 13.5 at 30^0 C
	: 6.4 – 18.7 at 37^0 C
U/10^{12} RBC's	: 146 – 376 at 30^{0}C
	: 202 – 522 at 37^0 C

Note: In case of low value of G6PDH activity, measure absorbance change for 5 min after addition of buffered substrate and divide by 5 to obtain ΔA/min and calculate the test results.

BIBLIOGRAPHY:

- Diagnostic Hematology by Rodak W.B Saunders 1995 ED: 218.
- Tietz, Clinical Chemistry, Saunders (986), page No. 1501-12
- Jocques Walloch, Interpretation of Diagnostic Tests, V Edition Page 315

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	Attention,see instructions for use		Consult Instructions For Use
IVD	For in vitro diagnostic use only	REF	Catalog #
2°C 8°C	Store between 2-8°C	LOT	Lot Number
	Do not use if package is damaged		Date of Manufacturing
	Manufacturer		Use by