ESTROM G-6-PD (Dye Decolourization Method)

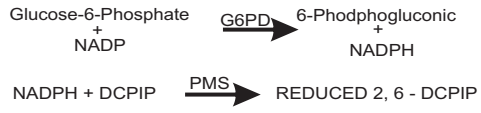
GLUCOSE - 6 - PHOSPHATE DEHYDROGENASE

ANGSTROM BIOTECH PVT. LTD

This reagent kit is for qualitative estimation of GLUCOSE-6 PHOSPHAT E DEHYDROGENASE activity in erythrocytes.

PRINCIPLE:

The substrate Glucose-6-Phosphate is oxidized to 6 phosphogluconate with the reduction NADP to NADPH. Reaction is catalysed by Glucose-6- Phosphate dehydrogenase that is present in erythrocytes. Reduction of NADP to NADPH decolourises the blue coloured indophenol dye (DCPIP) to a brick red colour which is contributed by the h emolysate. This reagent uses a potent inhibitor to 6-Phosphogluconate dehydrogenase thus preventing its interference, in the estimation of G6PD



CLINICAL SIGNIFICANCE :

Estimating G6PDH activity is a first important step in screening hemolytic episodes and anemia. Hereditary abnormalities can b e the cause of enzyme deficiencies.

Subject deficient in this enzyme are prone to hemolytic episodes and hemolytic anemias when treated with certain anti-malaria or sulfa drugs.

REAGENTS:

All the reagents are to be stored at 2 -8° c

	No. of bottles	
	8x0.5ml	15x0.5ml
Reagent 1 (Enzyme/coenzyme)	8	15
Reagent 2 (Buffer)	1	1
Reagent 3 (Lysing Reagent)	1	1
Reagent 4 (Inert oil)	1	1

SPECIMEN COL LECTION AND STORAGE :

* Fresh whole blood is necessary.
* Sample should be collected preferably in EDTA as an Anticoagulant.
* The hemoglobin content of the sample should be Estimated.

PRECAUTION:

* Estrom G-6-PDH reagent is for In Vitro diagnostic use Only.

REAGENT RECONSTITUTION:

(A) 8x0.5 ml & 15x0.5 ml: Reconstitute 1 vial of Reagent 1(Enzyme/Chromogen) using 0.5 ml of Reagent 2 (Buffer). The reagent should be reconstituted just befor e use and should be protected from light.

REAGENT STORAGE AND STABILITY:

All the reagents are stable up to expiry date stated on the label.

PROCEDURE:

Allow the sample and reagent to attain room temperature prior to use. Determine the hemoglobin content of the blood Sample

Dispense into test tubes	Test
Reagent 3 (Precooled Lysing Reagents)	1.0 ml
Sample (Fresh Whole Blood)	20 µl

Mix well and keep at 2-8° c for 5 minutes.

***Note**: if the hemoglobin content of the sample is significantly less/more than 15gms/dl, the sample volume may be adjusted as follows.*

Hemoglobin Concentration	Sample Volume
13 gms/dl	23 µl
14 gms/dl	21 µl
16 gms/dl	19 µl

PROCEDURE:

1. The hemolysate prepared as stated above should be transferred completely to the freshly prepared working reagents Mix well
2. Overlay 1 ml (approximately) of Reagent 4(Inert oil) to the above mixture
3. Close the vial immediately and incubate at 37° c.
4. Observe the change in the original blue colour of the working reagent to the brick red colour due to the hemolysate

OBSERVATION:

1. Observe the change in colour of the reac tion mixture after 30 minutes.
2. If the sample does not show decolourization, note for change in colour for every 5-10 minutes, till the decolourization is complete.
3. If the sample does not decolourise even after 60minutes, observe the change in absorbance every 30 minutes and follow up to 4-8 hours.
4. Samples deficient in G-6-PDH may decolourise after 2-24 hours or may not decolourise at all.
5. Some samples may recolourise after decolourization , this should be ignored, the initial decolourization time should be noted
6. In case of heterozygous males or females who are carrier it is advisable to quantitatively estimate G -6-PD activity.

REFERENCE VALUES:

Normal Range: Decolourization time (at 37° c , Hb content 15 gms/dl) : 30-60 minutes, it is recommended that each laboratory establish its own reference values.

BIBLIOGRAPHY:

- BEUTLER, E, BLUME, K.G., KAPLAN, J.C., LOHAR, G.W., RAMOT, B. and VALENTINE,W. N.(1979) International Committee for Standardization in Hematology.
- DACIE V., LEWIS S. In., Practical Hematology, 7th Edition (1991), pg.204-212.

Angstrom Biotech Pvt.Ltd.
G1 - 1035, RIICO Industrial Area
Phase - III, Bhiwadi,
Alwar, Rajasthan.
Pin Code - 302019
Email: info@angstrombiotech.in
Website: www.angstrombiotech.in.
Customer Care Number - 9599194831

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