GLUCOSE - 6 - PHOSPHATE DEHYDROGENASE (G6PD)

(Qualitative Screening Method)

ORDER INFORMATION

CODE : DL3301 - 10 Tests

INTENDED USE :

This reagent kit is intended for "**in vitro**" qualitative determination of G6PDH deficiency in Red Blood Cells.

CLINICAL SIGNIFICANCE :

G6PD deficiency is an inherited condition in which the body doesn't have enough of the enzyme Glucose - 6 - phosphate dehydrogenase, which helps red blood cells (RBCs) function normally. This deficiency can cause **hemolytic anemia**, usually after exposure to certain medications, foods or even infection. Most people with G6PD deficiency don't have any symptoms, while others develop symptoms of anemia only after RBCs have been destroyed a condition called **hemolysis**. In these cases the symptoms disappear once the cause or trigger is removed. In rare cases G6PD deficiency leads to chronic anemia.

There are many screening nonspecific tests like osmotic fragility autohemolysis tests etc. 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 deficiencies by enzyme assays.

One of the common enzyme deficiencies for hemolytic anaemia is measurement of Glucose-6-Phosphate Dehydrogenase, by a quantitative enzyme assay.

PRINCIPLE :

Glucose-6-Phosphate Dehydrogenase present in hemolysate acts on substrates Glucose-6-Phosphate (G-6-PD) and NADP to give NADPH, this NADPH decolorizes the blue colored indophenol dye (DCPIP) in presence of PMS. It leaves behind color which is due to hemolysate. The rate of reaction is proportional to enzyme activity of (G6PD) present in Erythrocytes. The time required for decolorization is inversely proportional to enzyme activity in the hemolysate.

$$\text{G-6-PO4 + NADP} \xrightarrow{\text{G6PD}} \text{6-Phosphogluconate + NADPH}$$

$$\text{NADPH + DCPIP} \xrightarrow{\text{PMS}} \text{DCPIP (Reduced)}$$

REAGENT COMPOSITION :

Reagent 1:	Enzyme Reagent
Reagent 2:	Buffer Reagent
Reagent 3:	Lysing Reagent
Reagent 4:	Inert Oil

MATERIALS REQUIRED BUT NOT PROVIDED :

- Clean & Dry Glassware.
- Micropipettes & Tips.
- Colorimeter or Bio-Chemistry Analyzer.

STABILITY OF REAGENT :

All the reagents are stable up to expiry date stated on the label when stored at temperature stated on the label

SAMPLES :

Fresh Whole Blood. Sample should be collected preferably in EDTA. Heparin should not be used as it interferes with the Finger prick blood may used as a sample provided the hemoglobin content is close to 15gm%. For unknown sample, the hemoglobin content must first be estimated and aliquot of blood may be corrected for low hemoglobin content.

WORKING REAGENT :

Working Reagent 1: Reconstitute 1 vial of Reagent 1 (Enzyme Reagent) using 0.5 ml Reagent 2 (Buffer). This is Working Reagent 1.
The reagent should be reconstituted just before use. Shake well to allow complete dissolution and should be protected from light.

ASSAY PROCEDURE :

Estimate Hemoglobin Content (gm/dl) of Whole blood. For assay, Use the sample volume corresponds to Hemoglobin concentration as follows.

Hemoglobin concentratin (gm/dl)	Sample Volume
7.0 - 9.5 gms/dl	40 µl
9.6 - 11.5 gms/dl	30 µl
11.6 - 13.5 gms/dl	25 µl
13.6 - 15.0 gms/dl	20 µl

Allow the sample and reagent to attain room temperature prior to use. Prepare hemolysate as below.

Dispense into test tubes	Test
Reagent 3 (Pre cooled lysing Reagent)	1 ml
Sample (Fresh Whole Blood)	(Refer table above)

Mix well and keep at 2-4ºC (Refrigerator) for 10 - 15 minutes.
1. The hemolysate prepared as stated above should be transferred completely to the freshly prepared Working Reagent 1 and mix well.
2. Immediately, Overlay 1 ml of Reagent 4 (Inert oil) to the above mixture.
3. Close (airtight) the vial immediately and incubate at 37ºC.
4. Observe the change in the original blue color of the Working reagent to the brownish color.

OBSERVATION:

1. Observe the change in color of the reaction mixture after 30 min.
2. If the sample does not show decolorization, note for change in color for every 5 minutes (or shorter intervals), till the decolorization is complete.
3. If the sample does not decolorize even after 60 minutes, observe the change in color every 30 minute and follow up to 4-8 hours.
4. Samples deficient in G-6-PDH may decolorize after 2-24 hours.
5. Some samples may recolorize after decolorization, this should be ignored, the initial decolorization time should be noted.
6. In case of heterozygous males or females who are carries it is advisable to quantitatively estimate G-6-PD activity.

REFERENCE NORMAL VALUE :

Decolorization time (at 37°C,Hb content 15 gm/dl) : 30-60 min.
In G6PD deficient (heterozygous males and homozygous females) decolorization time: 2 - 24 hours.
It is recommended that each laboratory establish its own range.

NOTE:

Sample may give false normal result in a deficient subject if the recticulocyte count is high, as recticulocytes have a higher G6PD activity than adult red cells is of special importance if the test is carried out immediately after a hemolytic episode in a drug (primaquine or any such) sensitive subject. After initial 15 minutes ti is better to observe the reaction tube at an interval of 5 minutes or less, as some of the sample may reach the end point and then slowly turn blue again, due to re-oxidation of the dye. Observation of the color changes should be restricted to the reaction mixture below the layer of oil and not at the interphase. Vitamin C supplements or large amount of dietary intake of Vitamin C may interfere with the reaction. To find out the G6PD activity of heterozygous males or females (carriers) it is advisable to estimate G6PD activity quantitatively, although mosaicism is better shown under microscope by Cytochemical staining.

BIBLIOGRAPHY :

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