G6PD (QUALITATIVE METHOD)

Code	Product Name	Pack Size
VI001A	G6PD (QUALITATIVE METHOD)	12 T

BEACON

GLUCOSE - 6 - PHOSPHATE DEHYDROGENASE (G6PD)

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.

REACTION:

G-6-PO4 + NADP G6PD6-Phosphogluconate + NADPH

NADPH + DCPIP PMSDCPIP (Reduced)

REAGENTS:

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

MATERIAL REQUIRED BUT NOT PROVIDED:

-Clean & Dry Glassware
-Laboratory Glass Pipettes or Micropipettes & Tips.

SAMPLE:

Fresh Whole Blood
Sample should be collected preferably in EDTA. Heparin should not be used as it interferes with the reaction.
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.

PREPARATION OF WORKING REAGENT:

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

REAGENT STORAGE AND STABILITY

All the reagents are stable up to expiry date stated on the label.
Reconstitute Reagent 1 only before use.

PRECAUTIONS:

– Avoid Freezing
– Protect from Direct sun light
– G6-PDH Reagent is for In vitro diagnostic use only

PROCEDURE:

Estimate Hemoglobin Content (gm/dl) of Whole blood.
Note: If the hemoglobin content of the sample is significantly less / more than 15 gms/dl, the sample volume may be adjusted 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 tmeperature prior to use.
Detarmine the hemoglobin content of the blood sample.

Dispense into test tubes	**Test**
Reagent 3 (Pre cooled lysing Reagent)	1 ml
Sample (Fresh Whole Blood)	20 µl (Refer table above)

Mix well and kep at 2-4ºC (Referigerator) for 10 - 15 minutes.

1. The hemolysate prepared as stated above should be transfeered completely to the freshly prepared working reagent, immediately mix well.
2. Overlay 1 ml of Reagent 4 (Inert oil) to the above mixture.
3. Close (airtight) the vial immediately and incubate at 37ºC.
4. Obeserve 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 minutes
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 absorbance 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 VALUE:

Normal Range : Decolorization time(at 37°C,Hb content 15 gms/dl) :30-60 minutes, 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.

REFERENCES:

BEUTLER, E, BLUME, K.G., KAPLAN. J.C. LOHAR, G.W. RAMOT, B, and VALENTINE, W.N. (1979) International Committee for Standardization in Hematology. Recommended Screening test for Glucose-6-Phosphate Dehydrogenase (G-6-PD) deficiency. British Journal of Hematology, 43, 465. DACIE V., LEWIS S. Practial Hematology, 7th Edition (1991) Pg.204-212.

IVD

JAS-ANZ BSCIC ISO 9001 : 2015 BSCIC ISO 13485:2016

SYMBOLS USED ON LABELS

REF Catalogue Number

Manufacturer

See Instruction for Use

LOT Lot Number

CONT Content

Storage Temperature

Expiry Date

IVD In Vitro Diagnostics

BEA/24/G6P/VI/IFU-00