Atlas Medical CE

G6PD QUALITATIVE KIT

IVD For *in vitro* diagnostic and professional use only

2°C 8°C Store at 2-8˚C.

INTENDED USE

The G-6-PD assay is an enzymatic method for the qualitative determination of G-6-PD activity in dried blood spots and whole blood specimen. The test is intended for use as a screening method for red cell glucose-6-phosphate dehydrogenase deficiency in newborns and adults.

INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD or G6PDH) is a cytosolic enzyme that catalyzes the chemical reaction. This enzyme participates in the pentose phosphate pathway, a metabolic pathway that supplies reducing energy to cells (such as erythrocytes) by maintaining the level of the co-enzyme nicotinamide adenine dinucleotide phosphate (NADPH). The NADPH in turn maintains the level of glutathione in these cells that helps protect the red blood cells against oxidative damage from compounds like hydrogen peroxide. Of greater quantitative importance is the production of NADPH for tissues involved in biosynthesis of fatty acids or isoprenoids, such as the liver, mammary glands, adipose tissue, and the adrenal glands. G6PD reduces NADP+ to NADPH while oxidizing glucose-6-phosphate. Clinically, an X-linked genetic deficiency of G6PD predisposes a person to non-immune hemolytic anemia.

TEST PRINCIPLE

The G-6-PD Assay utilizes glucose-6-phosphate-dehydrogenase, which in the presence of NADP, catalyses the oxidation of glucose-6-phosphate to 6-phosphogluconate. The NADPH produced is directly proportional to the concentration of Glucose-6-phosphate dehydrogenase present in the sample. The NADPH produced fluoresces under long wave UV lamps, the intention of fluorescence being proportional to the activity of the enzyme in the sample.

The assay procedure is according to reaction described by Beutler.

$$\text{Glucose-6-P} + \text{NADP}^+ \xrightarrow{\text{G-6-PD}} \text{gluconate-6-P} + \text{NADPH} + \text{H}^+$$

The NADPH produced in the reaction fluoresces under long-wave UV-light. If there is a marked deficiency of this enzyme, or if G-6-PDH is lacking entirely, no fluorescence will be observed.

MATERIALS

MATERIALS PROVIDED

- **Reagent lyophilized:**

Contents of solution	Concentration in the test
Glucose-6-phosphate	1 mmol/l
NADP	0,75 mmol/l
GSSG (oxidized glutathione)	0,8 mmol/l
Saponin	0,2%
Tris(hydroxymethyl)-aminomethane	225 mmol/l, pH 7.8

- **Dilution buffer** is ready to use.
- **Filter paper**.(Optional)

MATERIALS NOT PROVIDED

- Timer.
- G-6-PDH Controls.
- Single or multichannel automatic pipettes.

PRECAUTIONS

- Wear protective clothing and disposable gloves when dealing with samples and reagents. Wash hands after operations.
- Do not use reagents beyond the labeled expiry date.
- Do not mix or use components from kits with different batch codes.
- The used tests, specimens and potentially contaminated materials should be discarded according to the local regulations.
- Dispose of all specimens and materials used to perform the test as bio hazardous waste.
- Follow the instructions for use carefully before testing.
- All reagents contain (0.1%) sodium azide which is toxic and can be absorbed through the skin. When drained, the drains should be thoroughly flushed with water.
- Don't use the kit if damaged or the glass vials are broken or leaking and discard the contents immediately.
- Don't use these reagents if the label is not available or damaged.
- Do not use any solutions that have become turbid or discolored.

PREPARATION AND STABILITY OF REAGENT SOLUTION

1. Dissolve the contents of reagent lyophilized bottle (one bottle) with dilution buffer (one bottle).
2. Reagents stable for four weeks at 2-8˚C or two months at -20˚C after reconstitution. Stable for 24 months lyophilized in unopened vial at 2-8°C (See Expiry Date on label).
3. Dilution buffer stable for 24 months at 2-8°C (See Expiry date on the label).

SAMPLE COLLECTION HANDLING AND STORAGE

A. DRIED BLOOD SPOTS (DBS):

1. Collect dried blood spots sample from the infant's heel or whole blood.
2. Apply a drop of blood on filter paper to absorbent and let dry completely.
3. After the sample is taken and the blood has dried, the cards must be stored at 2-8°C.
   NOTE: Spots not stored under these conditions gradually lose the enzyme activity due to heat inactivation, causing potential risk of misclassifying samples as screen-positive.

B. WHOLE BLOOD SPECIMEN:

Whole blood may be used instead of dried blood. Heparin, citrate, oxalate and EDTA are suitable anticoagulants. Do not freeze blood. It is recommended to store blood specimen in the refrigerator and use them within 3-4 days after collection. Use 0,005 ml (5 microliters) for the assay.

PROCEDURE

1. Punch out a disk of blood-stained paper (5 mm in diameter) (3 mm can also be used) and introduce into a vial (volume 1-3 ml). In case of using whole blood sample, transfer 5 µl of the blood sample to the vial.
2. Transfer 5 µl of each normal and deficient Control (not provided in the kit) to separate vials.
3. Transfer 0.1 ml of reagent solution to each vial.
4. Mix well then incubate for 10 minutes at 25ºC.
   NOTE: Test Solution (One blood disk or whole blood sample with reagent solution).
5. Transfer 0.01 ml of the test solution to a new filter paper.
6. When the filter paper is dry (approximately after 1 hour), view under a long-wave UV-lamp in a darkened room.

INTERFERING OF THE RESULTS-EXPECTED VALUES

- Normal Control will show strong fluorescence after 10 min incubation. Deficient control will show very weak or no fluorescence after 10 min incubation.
- Samples obtained from normal or slightly reduced G-6-PDH activity will show strong fluorescence. Failure to fluoresce after 10 minute incubation suggests a total or marked deficiency of G-6-PDH.
  Each sample is classified according to the obtained result as follows:
  Totally Deficient: Very weak or no fluorescence after 10 min incubation
  Partially Deficient: Weak to moderate fluorescence after 10 min incubation
  Normal: Strong fluorescence after 10 min incubation.

- It is highly recommended to repeat the examination of all the classified Deficient neonates after a period of 6 months.
- It is recommended that samples which have been determined as deficient or intermediate by this procedure be assayed by a quantitative G-6-PDH.

PERFORMANCE CHARACTERISTICS

1. Accuracy:

This kit was compared against the commercial kit and showed an excellent correlation of the results obtained (R -squared > 0.99).

2. Sensitivity:

In a study performed in our laboratory, we evaluated the G6PD residual enzymatic activity in two hundred and thirty three samples concomitantly using a quantitative kit and our qualitative kit .The values of the samples, as measured by the quantitative kit ranged from 0.1 U/g Hb to 13.8 U/g Hb. The fluorescence of the samples was compared to the values obtained and it was shown that the sensitivity of the kit is 2.5 U/g Hb when the assay is performed according to the instructions. Samples with lower activity would show the same fluorescence as a 2.5 U/g Hb sample.

3. Specificity:

The oxidation of glucose-6-phosphate by G6PDH is specific. Any nonspecific formation of NADPH due to oxidation of other substrates due to endogenous enzymes occurs during the elution time while the other substrates are exhausted.

4. QC Requirements:

It is not recommended to run the test at temperatures lower than 24°C.

5. Reproducibility

Normal, deficient, and intermediate samples were assayed three times over a period of several days. Results obtained for each of the samples were identical for the replicate assays.

6. Correlation

Two hundred and thirty three (233) samples including normal, intermediate and deficient enzyme levels were assayed simultaneously by G-P-PD Qualitative kit and Quantitative kit. All samples were identified similarly by the two test kits.

7. Interfering Substances:

The effects of bilirubin, triglyceride and protein, at concentrations which mimic severely icteric, lipemic, and abnormal protein specimens, were determined by spiking whole blood and preparing dried blood spot specimens.

Substance	Amount (mg/dL)	G6PD (U/gHb)	Recovery (%)
Unconjugated Bilirubin	0	17.77	
	40	16.15	90.87
Conjugated Bilirubin	0	17.77	
	40	17.18	96.66
Triglycerides (Liposyn II)	0	18.32	
	1000	17.49	95.46
gG	0	18.20	
	2500	17.73	97.43

The effect of these substances on the assay's results is marginal (2.57 – 9.13%) and will never lead to the misclassification of a positive (Deficient) sample.

LIMITATIONS OF THE EXAMINATION PREOCEDURE

Low results are not diagnostic per se of G-6-PD deficiency but indicate the need for further study of the newborn from which a presumptive screen positive sample was received.

1. In some forms of G-6-PDH deficiency, young erythrocytes manifest normal enzyme activity. Blood from patients who have just experienced a hemolytic crisis must first be treated by the procedure described by Herz et.al (4) to separate the older erythrocytes from the prevailing population of young ones. Use 0,005 ml of the suspension so obtained for the assay.
2. If the patient has received a blood transfusion this test is clinically significant only after 30 days have elapsed because the donor's erythrocytes generally manifest a normal G-6-PDH activity and can thus bias the result before the expiration of this time.

REFERENCES

1. Beutler E. Drug-induced hemolytic anemia and non-spherocytic hemolytic anemia. In Glucose-6-Phosphate Dehydrogenase (Yoshida A. and Beutler E., Eds) pp. 3-12, Academic, Orlando.
2. Beutler E. A series of new screening procedures for pyruvate kinase deficiency, glucose – 6 – phosphate dehydrogenase deficiency and glutathione reductase deficiency. *Blood* 1966;28:553-562.
3. Dow PA, Petteway MB, Alperin JB. Simplified method for G6PD screening using blood collected on filter paper. *Am J Pathol* 1974;61:333-336.
4. Herz F, Kaplan E, Scheye ES. Diagnosis of erythrocyte glucose-6-phosphate dehydrogenase deficiency in the negro male despite hemolytic crisis. *Blood* 1970;35:953-954.
5. Luzzatto L, Mahta A. Glucose 6-Phosphate Dehydrogenase Deficiency. In: Scriver CR, Beaudet AL, Sly WS, Valle D eds. The Metabolic and Molecular Basis of Inherited Disease. USA: McGraw-Hill Publishers, 1995:3367-3398.

ATLAS Medical GmbH
Ludwig-Erhard Ring 3
15827 Blankenfelde-Mahlow
Germany
Tel: +49 - 33708 – 3550 30
Email: Info@atlas-medical.com
Website: www.atlas-medical.com

PPI1897A01
Rev B (21.04.2022)

REF	Catalogue Number		Temperature limit
IVD	*In Vitro* diagnostic medical device		Caution
Σ	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
LOT	Batch code		Manufacturer
	Fragile, handle with care		Use-by date
	Manufacturer fax number		Do not use if the package is damaged
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry