Trinity Biotech

GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY

(Procedure No. 400)

REF	400K-100-5 x 20	5 x 20
REF	400K-100X	10 x 10

For other languages
Pour d'autres langues
Für andere Sprachen
Para otras lenguas
Per le altre lingue

Para outras línguas
Για τις άλλες λώσσες
For andre sprog

www.trinitybiotech.com

INTENDED USE

Trinity Biotech Glucose-6-Phosphate Dehydrogenase (G-6-PDH) reagents are for the qualitative, visual, colorimetric determination of G-6-PDH deficiency in red cells.

BACKGROUND AND PRINCIPLE OF TEST

A visual semi-quantitative procedure for measuring G-6-PDH, (EC 1.1.1.49) in red cells was developed by Motulsky and Campbell-Kraut,[1] using brilliant cresyl blue as an indicator. Subsequently, other workers[2,3] replaced the dye with dichlorophenol indophenol. This method serves as the basis for the **Trinity Biotech** procedure for estimating red cell G-6-PDH.

G-6-PDH released from lysed erythrocytes catalyzes the conversion of glucose-6-phosphate to 6-phosphogluconate with reduction of nicotinamide adenine dinucleotide phosphate (NADP) to NADPH. In the presence of phenazine methosulfate (PMS), NADPH reduces the blue dye, dichlorophenol indophenol, to the colourless form. The rate at which colour visually disappears in the reaction mixture is proportional to the G-6-PDH content of red cells.

Glucose-6-Phosphate + NADP $\xrightarrow{\text{G-6-PDH}}$ 6-Phosphogluconate + NADPH

NADPH + Blue Dye $\xrightarrow{\text{PMS}}$ NADP + Colorless Dye Complex

REAGENTS

GLUCOSE-6-PHOSPHATE DEHYDROGENASE SUBSTRATE,

Catalogue No. 400-5 x 10: Substrate for Five tests per assay vial (G-6-PDH Deficiency Substrate 5 x 10), 2.5 ml size.

Catalogue No. 400-10 x 10: Substrate for Ten tests per assay vial (G-6-PDH Deficiency Substrate 10 x 10), 5 ml size.

When reconstituted according to directions, contains approximately the following concentrations of active ingredients:

Glucose-6-Phosphate	4.6 mmol/L
NADP	0.13 mmol/L
Dichlorophenol indophenol	0.55 mmol/L
Also contains PMS.	

TRIZMA® BUFFER SOLUTION, Catalogue No. 400-4-50
TRIZMA, 0.3 mol/L, pH 8.5 (37°C). 1% Chloroform added as preservative.

MINERAL OIL, Catalogue No. 400-5-100

PRECAUTIONS:
G-6-PDH reagents are for "*in vitro* diagnostic use". Normal precautions exercised in handling laboratory reagents should be followed. If you feel unwell, seek medical advice (show label where possible). Wear suitable protective clothing, gloves and eye/face protection. Dispose of waste observing all local, state and federal laws.
For professional use only.
In case of damage, do not use.

PREPARATION:
G-6-PDH Substrate Solution is prepared by reconstituting Glucose-6-Phosphate Substrate vials with the volume of TRIZMA Buffer Solution, Catalogue No. 400-4-50, indicated on reagent box. Leave reconstituted substrate standing for 10 minutes, to ensure it is fully reconstituted. Mix by gentle inversion. Before removing cap, tap vial on table surface to consolidate material on bottom of container.

Allow components to come to room temperature before use.

STORAGE AND STABILITY:
Note: Store unopened kit in refrigerator (2-8°C)

Store G-6-PDH Substrate vials at 2-8°C until the expiry date printed on the vial label. Substrate is light sensitive so avoid exposing vials to direct sunlight or bright lights during use. The expiration date is printed onto the reagent box label.
Reconstituted G-6-PDH Substrate Solution is stable 8 hours refrigerated (2-8°C) when protected from light.

Store TRIZMA Buffer Solution tightly capped in the refrigerator (2-8°C) until the expiry date printed on the vial label. Discard if there is visible evidence of microbial growth.

Store Mineral Oil at 2-26°C.
Reagent label is printed with the expiration date.

SPECIMEN COLLECTION AND STORAGE

It is recommended that specimen collection be carried out in accordance with CLSI document M29-A4.[4] No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious. For collection and storage of samples, follow the guidelines below:

- Whole blood is collected using EDTA[5] or heparin[6] as anticoagulant.
- G-6-PDH activity in whole blood may decline if sample is refrigerated (2-8°C), but may still fall within the normal range after a week or more at this temperature.
- If stored samples exhibit abnormally low results, the test should be repeated with freshly drawn blood.
- Freezing samples is not recommended and haemolysates should not be stored.[6]

INTERFERING SUBSTANCES:
Excessive numbers of leukocytes or platelets, which are rich in G-6-PDH, may interfere with assay.[7] In cases of anaemia, leukemia or leukocytosis, the buffy coat should be removed before preparing red-cell haemolysate.

Reticulocytes have higher G-6-PDH levels than mature erythrocytes. Therefore, assays should not be performed using samples collected after an individual has suffered a severe haemolytic crisis. In such cases, G-6-PDH levels may appear falsely elevated. Under these conditions, detection of deficiency may require family studies. The screening test should only be performed after mature red cell levels have returned to normal.[8] Certain drugs and other substances are known to influence circulating levels of G-6-PDH.[9]

PROCEDURE

MATERIALS PROVIDED:
See "Reagents" section.

MATERIALS/REAGENTS REQUIRED BUT NOT PROVIDED:
- Deionized water
- Pipettes to reliably deliver 0.05, 0.5, 1.0 and 5.0 ml
- Timer
- Water bath at 37°C
- **G-6-PDH CONTROLS**
  G-6-PDH Control Normal, 6 x 0.5 ml, G6888
  G-6-PDH Control Intermediate, 6 x 0.5 ml, G5029
  G-6-PDH Control Deficient, 6 x 0.5 ml, G5888

Lyophilized preparations containing G-6-PDH in a stabilized human red cell haemolysate base.

G-6-PDH Controls are POTENTIALLY BIOHAZARDOUS MATERIALS. Source materials from which these products were derived were found negative for HBsAg and for antibodies against HCV and HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, these products should be handled observing the same safety precautions employed when handling any potentially infectious material.

Quality controls should return their expected result; otherwise the test run is invalid.

PROCEDURE:
1. To prepare red cell haemolysate, add 0.05 ml blood to 2.5 ml water. Shake gently and allow to stand for about 5 minutes.
   Note: If the haemoglobin (Hb) content is much above or below 15 g/dl, the following table may be used to determine the appropriate volume of water to add to 0.05 ml blood to prepare the haemolysate.

Hb (g/dl)	9	12	15	18	21
Volume of water (ml)	1.5	2.0	2.5	3.0	3.5

2. Reconstitute Glucose-6-Phosphate Substrate vials with volume of TRIZMA Buffer Solution, Catalogue No. 400-4-50, indicated on the reagent box.
   a. For Five tests per assay vial (G-6-PDH Deficiency Substrate 5 x 10), Catalogue No. 400-5 x 10 add 2.5 ml of TRIZMA buffer solution.
   b. For Ten test per assay vial (G-6-PDH Deficiency Substrate 10 x 10), Catalogue No. 400-10 x 10 add 5.0 ml of TRIZMA buffer solution.
3. a. For Five tests per assay vial (G-6-PDH Deficiency Substrate 5 x 10), Catalogue No. 400-5 x 10: Pipette 0.5 ml G-6-PDH Substrate Solution into a tube labeled TEST, add 1.0 ml red cell hemolysate and shake gently to mix.
   b. For Ten test per assay vial (G-6-PDH Deficiency Substrate 10 x 10), Catalogue No. 400-10 x 10: Pipette 0.5 ml G-6-PDH Substrate Solution into a tube labeled TEST, add 1.0 ml red cell hemolysate and shake gently to mix.
   Note: ***Trinity Biotech*** *recommends test tubes approximately 13 to 16 mm in diameter.*
4. Promptly layer approximately 2 ml Mineral Oil, Catalogue No. 400-5-100 / 400-5-1000, on top of reaction mixture.
   Note: Do not agitate mixture after adding oil.
5. Place vial or tube in 37°C water bath in subdued light. Note time.
6. The TEST should be observed at 15-minute intervals up to 1 hour for a change in colour from its original deep blue to a maroon or reddish endpoint. DO NOT AGITATE the reaction mixture during the incubation. Introduction of air will cause the blue colour to reappear. For normal blood, the initial deep blue colour of the reaction mixture will gradually reach the endpoint within 20-60 minutes. The endpoint can be more readily detected if the vial or tube is viewed horizontally in front of a bright light. There may be a thin blue layer remaining at the oil/aqueous interface due to trapped air which has reoxidized reduced dye. This layer should be ignored when evaluating the endpoint. Viewing in front of a bright light will prevent the endpoint from being obscured by reflection from this blue coloured layer.

QUALITY CONTROL:
The reliability of test results should be monitored by the use of at least two levels of G-6-PDH controls with each series of assays. **Trinity Biotech** offers three G-6-PDH Controls for this purpose: Deficient (Catalogue No. G5888), Intermediate (Catalogue No. G5029) and Normal (Catalogue No. G6888). Completion times determined for these materials by this procedure should fall within the stated times of the controls.

LIMITATIONS

This test is designed to distinguish normal from grossly deficient samples and should not be used to assess the degree of deficiency. It is recommended that samples requiring longer than 60 minutes to reach the endpoint (indicating G-6-PDH deficiency) be assayed by a quantitative G-6-PDH technique such as **Trinity Biotech** Procedure No. 345-UV.

EXPECTED VALUES

Blood was collected from 100 G-6-PDH normal and 25 G-6-PDH deficient individuals and assayed according to the described method. As shown in the table, all normal samples reached the colour change endpoint within 60 minutes. All deficient samples failed to reach a colour change within 60 minutes.

Endpoint (minutes)	0-15	16-30	31-45	46-60	>60
Normal Samples	52	48	0	0	0
Deficient Samples	0	0	0	0	25

It should be noted that the expected values are based on incubation at 37°C. When other incubation temperatures are employed, the endpoint time may differ. For example, a normal specimen that reaches the endpoint in 30 minutes at 37°C may take longer at 25°C.

PERFORMANCE CHARACTERISTICS

REPRODUCIBILITY:
A series of 6 replicate determinations were performed on blood samples collected from both G-6-PDH normal and deficient patients at each of three time points over 5 days (18 replicates in total). Dye decolorization time was within 0-15 minutes for all normal G-6-PDH sample replicates, no dye decolorization was observed for the G-6-PDH deficient samples within 60 minutes.

CORRELATION:
The described visual colorimetric method is recommended because of its dependability in detecting G-6-PDH deficient individuals. A comparison of a sample cohort of 125 patient samples including 100 normal and 25 deficient enzyme level samples assayed by this G-6-PDH 400K Kit, the Trinity Biotech G-6-PDH 203A Kit and an approved quantitative test kit, has established its reliability.

TRIZMA is a registered trademark of Sigma-Aldrich Co., St. Louis, MO, USA.

BIBLIOGRAPHY

1. Motulsky AG, Campbell-Kraut JM: Population genetics of glucose-6-phosphate dehydrogenase deficiency of the red cell. IN Proceedings of the Conference on Genetic Polymorphisms and Geographic Variations in Disease. B Blumberg, Editor, Grune & Stratton, New York, 1962, p 159.
2. Bernstein RE: Rapid screening dye test for detection of glucose-6-phosphate dehydrogenase deficiency in red cells. Nature 194: 192, 1962.
3. Ells HA, Kirkman HN: A colorimetric method for assay of erythrocytic glucose-6-phosphate dehydrogenase. Proc Soc Exper Biol Med 106:607, 1961.
4. Clinical and Laboratory Standards Institute (CLSI). Protection of laboratory workers from occupationally acquired infections; Approved guideline—Fourth Edition. CLSI document M29-A4 Vol. 34, No. 8, 2014.
5. Dawson JP, Thayer WW, Deforges JF: Acute hemolytic anemia in the newborn infant due to naphthalene poisoning: Report of two cases with investigations into the mechanism of the disease. Blood 13:113, 1958.
6. Kachmar JF: Enzymes. IN Fundamentals of Clinical Chemistry. NW Tietz, Editor, Saunders, Philadelphia 1970, pp 453-458.
7. Echler G: Determination of glucose-6-phosphate dehydrogenase. Am J Med Technol 49:259, 1983.
8. Glucose-6-Phosphate Dehydrogenase Deficiency. IN Hematology, WJ Williams, E Beutler, AJ Erslev, RW Rundles, Editors, McGraw-Hill, 1972, p 397.
9. Young DS: Effects on drugs on clinical laboratory tests, 3rd ed. AACC Press, Washington (DC), 1990; Supplement No. 1, 1991.

ORDERING INFORMATION

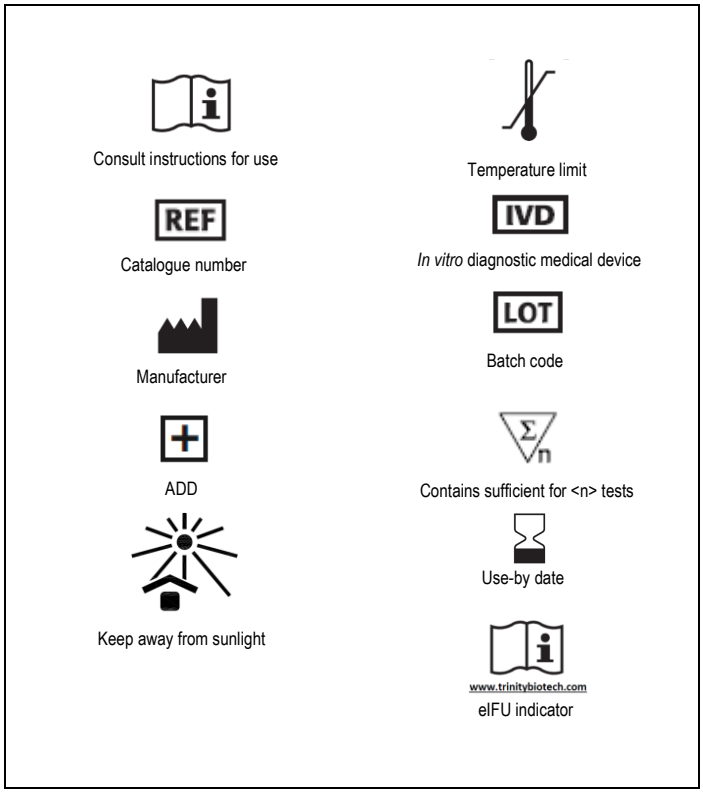
KITS

Catalogue No.	**400K-100-5 x 20**	**400K-100X**
Maximum Assays	100	100
Each Contains		
Description(Catalogue No.)	**400K-100-5 x 20**	**400K-100X**
Substrate (400-5 x 10)	2 x 10 vials	0 vials
Substrate (400-10 x 10)	0 vials	10 vials
TRIZMA® Buffer (400-4-50)	50 ml	50 ml
Mineral Oil (400-5-100)	2 x 100 ml	2 x 100 ml

INDIVIDUAL REAGENTS

Catalogue No.	**Item**	**Quantity**
400-5 x 10 (5 assays/vial)	Deficiency Substrate	10 x 2.5 ml
400-10 x 10 (10 assays/vial)	Deficiency Substrate	10 x 5 ml
400-4-50	TRIZMA® Buffer	50 ml
400-5-100	Mineral Oil	100 ml
400-5-1000	Mineral Oil	1000 ml
Catalogue No.	**Item**	**Quantity**
G 6888	Normal	6 x 0.5 ml
G 5029	Intermediate	6 x 0.5 ml
G 5888	Deficient	6 x 0.5 ml

GUIDE TO SYMBOLS



Trinity Biotech plc,
IDA Business Park
Southern Cross Road
Bray, Co. Wicklow
Ireland
Tel. 353 1 2769800
Fax. 353 1 2769888
www.trinitybiotech.com

USA ENQUIRIES:
Trinity Biotech USA
2823 Girts Road
Jamestown, NY 14701
Tel: 1800 325 3424
Fax: 716-488-1990

CE