Trinity Biotech

GLUCOSE-6 PHOSPHATE DEHYDROGENASE (G-6-PDH)

REF 345

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INTENDED USE

Trinity Biotech Glucose-6-Phosphate Dehydrogenase reagents are for the quantitative, ultraviolet, kinetic determination of G-6-PDH in blood at 340 nm.

SUMMARY

Glucose-6-phosphate dehydrogenase (G-6-PDH, D-glucose-6-phosphate: oxidoreductase, EC 1.1.1.49) catalyzes the first step in the pentose phosphate shunt, oxidizing glucose-6-phosphate (G-6-P) to 6 phosphogluconate (6-PG) and reducing NADP to NADPH. The **Trinity Biotech** procedure is a modification of the spectrophotometric methods of Kornberg and Horecker [1] and of Lohr and Waller,[2] involving the following reaction:

$$G\text{-}6\text{-}P + NADP^+ \xleftarrow{G\text{-}6\text{-}PDH} 6\text{-}PG + NADPH + H^+$$

Nicotinamide adenine dinucleotide phosphate (NADP) is reduced by G-6-PDH in the presence of G-6-P. The rate of formation of NADPH is proportional to the G-6-PDH activity and is measured spectrophotometrically as an increase in absorbance at 340 nm. Production of a second molar equivalent of NADPH by erythrocyte 6-phosphogluconate dehydrogenase (6-PGDH) according to the reaction:

$$6\text{-}PG + NADP^+ \xleftarrow{6\text{-}PGDH} Ribulose\text{-}5\text{-}Phosphate + NADPH + H^+ + CO_2$$

is prevented by use of maleimide, an inhibitor of 6-PGDH.

REAGENTS

G-6-PDH REAGENT
Catalogue No. 345-5: Five-Assay Vial, 5.5 ml size
Reconstituted reagent will contain NADP, 1.5 mmol/L, and maleimide, 12 mmol/L. Also contains buffer, stabilizer and lysing agent.

G-6-PDH SUBSTRATE SOLUTION, Catalog No. 345-8
Glucose-6-phosphate, 1.05 mmol/L, buffer and magnesium salt. Sodium azide, 0.1%, added as preservative.

PRECAUTIONS:
G-6-PDH reagents are for "in vitro diagnostic use". Normal precautions exercised in handling laboratory reagents should be followed. Dispose of waste observing all local, state and federal laws.

G-6-PDH Substrate Solution contains sodium azide which may react with lead and copper plumbing to form highly explosive metal azides. Avoid azide accumulation.

For professional Use Only
In case of damage, do not use.
If turbidity is observed in the substrate or reconstituted reagent, do not use.

PREPARATION:

G-6-PDH ASSAY SOLUTION is prepared by reconstituting G-6-PDH Reagent vial with volume of deionized water indicated on vial label or application sheet. Swirl gently and invert several times to dissolve contents. Wait 2–3 minutes and mix again.
G-6-PDH SUBSTRATE SOLUTION is supplied ready for use.

STORAGE AND STABILITY

Store unopened G-6-PDH Reagent vials and the G-6-PDH Substrate Solution in refrigerator (2–8°C). Reagents are stable until expiration dates shown on the labels.
Reconstituted G-6-PDH Assay Solution is stable for 8 hours at room temperature (18–26°C) or 28 days refrigerated (2–8°C).

OPTIONAL REAGENTS

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

Deficient Level, Catalogue No. G 5888
Intermediate Level, Catalogue No. G 5029
Normal Level, Catalogue No. G 6888

RED CELL LYSING REAGENT, Catalogue No. R 1129 Saponin, 0.2%. For use with discrete analyzer applications.

POTASSIUM DICHROMATE

A potassium dichromate solution is recommended as replacement for water in the reference cell in order to bring the absorbance reading of test within a range of greater accuracy when doing manual assays.

SPECIMEN COLLECTION AND STORAGE

It is recommended that specimen collection be carried out in accordance with CLSI document M29-A4. 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.

Whole blood collected with ethylenediaminetetraacetic acid (EDTA), heparin or acid-citrate-dextrose (ACD) is satisfactory.[3-7] Red cell G-6-PDH is stable in whole blood for one week refrigerated (2–8°C), but is unstable in red cell haemolysates.[8] Freezing of blood is not recommended.[3]

Since activity is reported in terms of number of red blood cells or grams haemoglobin, the red cell count or haemoglobin concentration should be determined prior to performing the G-6-PDH assay. The integrity of erythrocytes collected in ACD is preserved even after prolonged storage so that obtaining accurate red cell counts usually poses no problem.[5] However, red cell counts on specimens collected in heparin become unreliable after about 2 days.[5] Thus, for heparinized samples, results are best reported in terms of haemoglobin concentration.

INTERFERING SUBSTANCES:

Both copper, which completely inhibits the enzyme at a concentration of 100 μmol/L, and sulfate ions (0.005 mol/L) will decrease observed levels of G-6-PDH activity[14]. Certain drugs and other substances are known to influence circulating levels of G-6-PDH.[9] Sulfasalazine and sulfapyridine may cause interference with test results.
Reticulocytes have higher G-6-PDH levels than mature red cells. Therefore it is not recommended that assays be performed after a severe haemolytic crisis, since G-6-PDH levels may appear falsely elevated. Under those conditions, detection of deficiency may require family studies. Testing may be more helpful after the level of mature red cells has returned to normal. Under normal circumstances, activity contributed by leukocytes, platelets and serum is relatively small. However, in cases of extreme anaemia, grossly elevated white counts or very low levels of red cell G-6-PDH activity, the contribution to the total made under these conditions may be significant. See ''Use of Buffy-Coat-Free Samples'' section.

DISCRETE ANALYZER APPLICATIONS

Application procedures using G-6-PDH reagents are available for various automated instruments. Please contact **Trinity Biotech** Technical Services Department for more information.

PROCEDURE

MATERIALS PROVIDED

See ''Reagents'' section.

MATERIALS REQUIRED BUT NOT PROVIDED

Spectrophotometer, capable of accurately measuring absorbance at 340 nm, with temperature controlled cuvette compartment (Water bath or incubator may be used instead).
Pipeting devices for accurate delivery of volumes required for the assay.
Cuvettes with optical properties suitable for use at 340 nm.
Equipment and reagents for performing a red cell count or for determining haemoglobin concentration are also required.

PROCEDURE

The temperature of the reaction mixture should be maintained at 30°C or some other constant temperature (see ''Temperature Correction'' section).

1. Using Five-Assay Vial, Catalog No. 345-5
   a. To cuvette labeled TEST, add 1.0 ml G-6-PDH Assay Solution.
   b. Add 0.01 ml blood and mix thoroughly to completely suspend erythrocytes. Let stand at room temperature (18–26°C) for 5–10 minutes.
   c. Add 2.0 ml G-6-PDH Substrate Solution and mix gently by inverting several times. Proceed to step 2.
2. Place cuvette in constant temperature cuvette compartment or water bath and incubate for approximately 5 minutes to attain thermal equilibrium.
3. Read and record absorbance (A) of TEST at 340 nm vs water or Potassium Dichromate Solution. This is INITIAL A. (If using a water bath or incubator, return cuvette to it.)
4. Exactly 5 minutes later, again read and record absorbance. This is FINAL A.
5. To determine G-6-PDH activity, refer to ''Calculations'' section.

CALIBRATION:

The procedure is standardized on the basis of the millimolar absorptivity of NADPH, which is 6.22 at 340 nm. The oxidative conversion of G-6-P by G-6-PDH leads to reduction of NADP to NADPH on a molar equivalent basis. Measurement of the rate of increase in absorbance (ΔA) at 340 nm serves to quantitate enzymatic activity. The maximum G-6-PDH activity which may be measured by this procedure is approximately 650 U/10[12] RBC or 19.5 U/g Hb.

QUALITY CONTROL:

Reliability of test results should be monitored by use of normal and abnormal control materials within each run. **Trinity Biotech** Glucose-6-Phosphatase Dehydrogenase Controls such as the following are suitable for this purpose: Deficient, Catalogue No. G5888; Intermediate, Catalogue No. G5029; and Normal, Catalogue No. G6888. A control range should be established by the laboratory to determine the allowable variation in day to day performance for each level of control.

Controls falling outside the upper or lower limit of the established ranges indicate the assay may be out of control. Failure to meet Quality Control specifications should be investigated and resolved. Recalibration is suggested whenever control materials are not within the established acceptable range. The assay should then be repeated. If problems cannot be resolved, contact **Trinity Biotech** Technical Services.

CALCULATIONS

$$\Delta A \text{ per min} = \frac{\text{FINAL A} - \text{INITIAL A}}{5}$$

G-6-PDH activity is expressed as U/10^{12} erythrocytes (RBC) or as U/g haemoglobin (Hb).

$$\text{G-6-PDH (U/}10^{12}\text{ RBC)} = \frac{\Delta A \text{ per min} \times 3.01 \times 10^{12} \times TCF}{0.01 \times 6.22 \times (N \times 10^{6}) \times 1000}$$

Where:

3.01	=	Total reaction volume (ml)
10^{12}	=	Factor for expressing activity in 10^{12} cells
0.01	=	Sample volume (ml)
6.22	=	Millimolar absorptivity of NADPH at 340 nm
N x 10^{6}	=	Red cell count (red cells/mm^3) determined for each specimen
1000	=	Conversion of red cell count from mm^3 to ml
TCF	=	Temperature correction factor (1 at 30°C)

This equation reduces to:

$$\text{G-6-PDH (U/}10^{12}\text{ RBC)} = \Delta A \text{ per min} \times \frac{48{,}390}{N} \times TCF$$

Where:

N	=	Red cell count divided by 10^{6}
TCF	=	Temperature correction factor (1 at 30°C)

$$\text{G-6-PDH (U/g Hb)} = \Delta A \text{ per min} \times \frac{100 \times 3.01}{0.01 \times 6.22 \times \text{Hb (g/dl)}} \times TCF$$

$$= \Delta A \text{ per min} \times \frac{4839}{\text{Hb (g/dl)}} \times TCF$$

Where:

100	=	Factor to convert activity to 100 ml
3.01	=	Total reaction volume (ml)
0.01	=	Sample volume (ml)
6.22	=	Millimolar absorptivity of NADPH at 340 nm
Hb (g/dl)	=	Haemoglobin concentration determined for each specimen
TCF	=	Temperature Correction Factor (1 at 30°C)

EXAMPLE:

Assay of a specimen which had a red cell count of 4.6 x $10^{6}/mm^3$ and a haemoglobin concentration 15.2 g/dl resulted in a ΔA per min at 30°C of 0.028.

$$\text{G-6-PDH (U/}10^{12}\text{ RBC)} = 0.028 \times \frac{48{,}390}{4.6} = 295$$

$$\text{G-6-PDH (U/g Hb)} = 0.028 \times \frac{4839}{15.2} = 8.9$$

Note: If ΔA per min is greater than 0.060, repeat determination using 5 µl blood and multiply results by 2.

USE OF BUFFY-COAT-FREE SAMPLE

Under normal circumstances G-6-PDH activity contributed by leukocytes, platelets and serum is relatively small. However, as reported by Echler[10] and others,[11] more accurate measurement of red cell G-6-PDH activity, especially in the presence of anemia and/or leukocytosis, can be achieved by using buffy coat-free blood samples for assay. Thus, in case of a borderline value obtained with whole blood, it may be warranted to repeat the assay on a buffy coat-free sample.

TEMPERATURE CORRECTION

When temperature is 30°C, no temperature correction factor (TCF) is required in the calculations. If assay is performed at a temperature other than 30°C, a TCF must be used.

Cuvette Temperature (°C)	TCF	Cuvette Temperature (°C)	TCF
20	1.90	30	1.00
21	1.76	31	0.94
22	1.66	32	0.89
23	1.55	33	0.83
24	1.46	34	0.78
25	1.37	35	0.74
26	1.28	36	0.70
27	1.20	37	0.66
28	1.13	38	0.62
29	1.06	39	0.58

UNIT DEFINITION

One International Unit (U) is that amount of G-6-PDH activity that will convert 1 micromole of substrate per minute under the conditions specified in this insert. Activity may be expressed in terms of either a standard number of cells or amount of haemoglobin. Since it is the G-6-PDH activity per cell that is important, the former convention is preferred, despite the fact that it is believed by some that red cell counts are subject to considerable uncertainty.[3] Haemoglobin concentration may be determined with greater accuracy, but the amount of haemoglobin contained in a cell is under separate genetic control and may vary independently of G-6-PDH activity.[3]

EXPECTED VALUES

The expected range of G-6-PDH activity is dependent on the measurement method, a previous study using this test on samples obtained from 90 clinically healthy adult males and females, measured at 30°C determined an expected G-6-PDH activity range of 4.6-13.5 U/g HB or 146-376 U/10^{12} RBC. A further publication determines the median G-6-PDH activity range between 8.0 and 12.0 U/g HB[13].

Values for newborns may range somewhat higher. It is strongly recommended that each laboratory establish an expected range, characteristic for the local population.

It has been determined that G-6-PDH deficiency in red cells is the basis for certain drug-induced haemolytic anemias.[12] This type of susceptibility to drug-induced haemolysis is often called ''primaquine sensitivity'' because studies which led to its characterisation were made during investigations of the haemolytic properties of this antimalarial compound.

PERFORMANCE CHARACTERISTICS

REPRODUCIBILITY

The following was performed across three clinical chemistry analysers.
Eleven replicate assays of two samples (of G-6-PDH activities ranging from 15-70 U/dL) tested on a single day yielded coefficients of variation of 0.71% to 3.05%.

Eleven replicate assays of two samples (of G-6-PDH activities ranging from 15-70 U/dL) tested on each of three separate days yielded coefficients of variation of 1.5% to 12.4%.

SENSITIVITY

Assuming the limit of sensitivity to represent a change in absorbance at 340 nm of 0.001 per minute, a G-6-PDH activity of 0.4 U/g Hb or 10.8 U/10^{12} RBC may be detected using this procedure (assuming a haemoglobin concentration of 12.0 g/dl and a red cell count of 4.5 x $10^{6}/mm^3$).

SPECIFICITY

The oxidation of glucose-6-phosphate by G-6-PDH is specific. Any nonspecific formation of NADPH due to oxidation of other substrates by endogenous enzymes occurs during the preincubation period. 6-Phosphogluconate dehydrogenase is completely inhibited by maleimide in the reagent system,[2] preventing formation of additional NADPH which might otherwise occur through oxidation of the 6-phosphogluconate produced in the initial reaction.

CORRELATION

This test kit was correlated against an IVD of equivalent intended use on two different clinical chemistry analyser systems.

System 1:

"Results obtained by the method described in this procedure were compared with those obtained when the same 27 specimens were assayed by a similar procedure. Comparison of these data yielded a linear regression equation with y = 1.09x + 0.1 and a correlation coefficient of 0.999".

System 2:

"Results obtained by the method described in this procedure were compared with those obtained when the same 27 specimens were assayed by a similar procedure. Comparison of these data yielded a linear regression equation with y = 1.10x + 0.33 and a correlation coefficient of 0.992".

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ORDERING INFORMATION

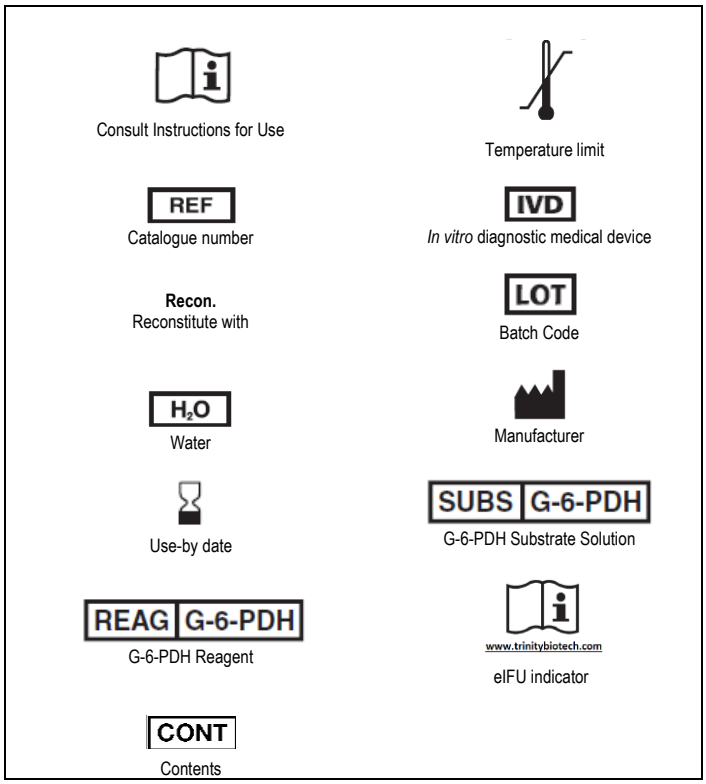
KIT	
Catalogue No.	**345-B**
Maximum Assays (manual method)	50
Contents – Catalogue Numbers	
G-6-PDH Reagent, 345-5	10 x 5.5 ml
G-6-PDH Substrate Solution, 345-8	2 x 50 ml
G-6-PDH Reagents are available in kits only and cannot be purchased separately.	

OPTIONAL REAGENTS

Catalogue No.	Item	Quantity
G-6-PDH CONTROLS	G-6-PDH CONTROLS	
G 5888	Deficient Level	6 x 0.5 ml
G 5029	Intermediate Level	6 x 0.5 ml
G 6888	Normal Level	6 x 0.5 ml
R 1129*	Red Cell Lysing Reagent	4 x 25 ml

*For discrete analyzer applications

GUIDE TO SYMBOLS



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