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 Walter 2 involving the following reaction:

G-6-P + NADP\(^+\) \rightarrow 6-PG + NADPH + H\(^+\) + CO\(_2\)

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-PGDH + ribulose-5-phosphate + NADPH + H\(^+\) + CO\(_2\)

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

REAGENTS

G-6-PDH REAGENT

Catalogue No. 345-1: Single Assay Vial, 1.0 ml size
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, Catalogue 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 Reagent is HARMFUL. May cause sensitization by inhalation and skin contact. Wear suitable protective clothing.

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.

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 5 days refrigerated (2-8°C).

OPTIONAL REAGENTS

G-6-PDH CONTROLS

Lipophilized controls containing G-6-PDH in a stabilized red cell haemolysate base.

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

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

POTASSIUM DICROMATE

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-A3. 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. 3,4 Red cell G-6-PDH is stable in whole blood for one week refrigerated (2-8°C), but is unstable in red cell haemoglobin. 5 Freezing of blood is not recommended. 5

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. 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. Certain drugs and other substances are known to influence circulating levels of G-6-PDH. 6

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). Pipetting 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. Prepare reaction mixture:

Using Single Assay Vial, Catalog No. 345-1
a. Add 0.01 ml blood directly to vial containing G-6-PDH Assay Solution and mix thoroughly to completely suspend erythrocytes. Let stand at room temperature (18-26°C) for 5-10 minutes.

b. Add 2.0 ml G-6-PDH Substrate Solution directly to vial and mix gently by inverting several times.

c. Transfer contents of vial to cuvette labeled TEST and proceed with step 2.

OR

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 (18-28°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 E.)

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 absorbivity 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\(^5\) RBC or 15.5 μg 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. Trinity Biotech Reliant C program may also be used to monitor the assay performance.

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
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 diaphorization period. 6-Phosphogluconate dehydrogenase is completely inhibited by maleimide in the reagent system,7 preventing formation of additional NADPH which might otherwise occur through oxidation of the 6-phosphogluconate produced in the initial reaction.

**Correlation**

Results obtained by the method described in this procedure were compared with those obtained when the same 27 specimens were assayed by a modification of the method of Marks.13 Comparison of these data yielded a linear regression equation with y = 1.47x − 0.26 and a correlation coefficient of 0.951.8

**References**

6. Bishop C: Assay of glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and 6-phosphogluconate dehydrogenase activity in red cells. J Lab Clin Med 67: 149, 1966

**ORDERING INFORMATION**

<table>
<thead>
<tr>
<th>Catalogue No.</th>
<th>345-A</th>
<th>345-B</th>
</tr>
</thead>
<tbody>
<tr>
<td>KIT</td>
<td>20</td>
<td>50</td>
</tr>
</tbody>
</table>

**Contents - Catalogue Numbers**

| G-6-PDH Reagent, 345-1 | 20 x 1 ml - |
| G-6-PDH Reagent, 345-5 | 10 x 5 ml |
| G-6-PDH Substrate Solution, 345-B | 50 ml |

**Red Cell Lyzing Reagent**

| G 1125 | 4 1/25 ml |

*For discrete analyzer applications.