Glucose Color Reagent

**Order No.** | **Description**
---|---
R80038 | 5 x 100 mL
R80039 | 5 x 500 mL

**INTENDED USE**
For the quantitative in vitro determination of glucose in serum.

**TEST SUMMARY**
The reagent requires a single absorbance reading. This method for the determination of glucose employs glucose oxidase (GOD) and a modified Trinder (1) color reaction, catalyzed by the enzyme peroxidase. Glucose is oxidized to D-gluconate by GOD with the formation of an equimolar amount of hydrogen peroxide. In the presence of peroxidase (HPDO), 4-aminoantipyrine (4-AAAP) and p-hydroxybenzene sulfonate (p-HBS) are oxidatively coupled by hydrogen peroxide to form a quinoneimine dye, intensely colored in red. The intensity of color in the reaction solution is proportional to the concentration of glucose in the sample.

This assay is based on the following reactions:

\[
\text{C}_6\text{H}_12\text{O}_6 + \text{H}_2\text{O} \rightarrow \text{D-Gluconate} + \text{H}_2\text{O}_2
\]

**REAGENT COMPOSITION**
The reagent is in single vial in the form of dry powder. When the reagent is dissolved in water as per directions, the solution will have the following approximate composition:

- **Reactive ingredients:**
  - 4-Aminoantipyrine: 0.6 mmol/L
  - Glucose oxidase (microbial): 20000 U/L
  - p-Hydroxybenzene sulfonate: 20 mmol/L
  - Peroxidase (plant): 6700 U/L

- **Non-reactive ingredients:**
  - Buffers, stabilizers and fillers

**REAGENT PREPARATION**
Dissolve the dry reagent in the vials with deionized or distilled water. Transfer the content of each vial into a suitable clean and dry container. Dissolve the dry reagent in 100 mL of water using a part of the water to rinse the vial. Dissolve the contents of the 500 mL vial (Product 80039) in 500 mL of water. In this case also use a part of the water to rinse the vial.

Store the reconstituted reagent in the refrigerator. Protect from strong light. Avoid contaminating the reagent.

**REAGENT STORAGE AND STABILITY**
The dry reagent in the original sealed container is stable until the expiration date on the vial, when stored in the refrigerator (2–8 °C).

The reconstituted reagent is stable at room temperature (20–25 °C) for at least 1 week and in the refrigerator (2–8 °C) for at least 1 month. If the freshly reconstituted reagent has an absorbance over 0.300 at 500 nm against water, discard the reagent. Do not use the reagent if it fails to recover stated values for control sera. Control sera with a very high bilirubin content should not be used. Do not use the reagent if it develops turbidity, since this may indicate contamination.

**PRECAUTIONS**
Good laboratory safety practices should be followed when handling any laboratory reagent. Refer to a recognized laboratory safety program for additional information. (See GP17-T, Clinical Laboratory Safety; Tentative Guideline (1994), National Committee on Clinical Laboratory Standards, Wayne, PA.)

Intended for in vitro diagnostic use only.

**SPECIMEN COLLECTION, PREPARATION AND STORAGE**
Avoid hemolysis in the collection of blood and in the separation of serum or plasma from the formed elements. Enzymes released from the erythrocytes will utilize glucose, with resulting false low results. Catalase from the erythrocytes will compete with peroxidase for hydrogen peroxide, again giving erroneously low results.

Serum or heparin plasma should be used. Separate from blood as soon as possible. Store in refrigerator, but for not longer than 24 hours. For longer period store frozen. Mix well after thawing and before assay.

**INTERFERING SUBSTANCES**
Szasz et al. (2) examined the effect of 43 drugs on glucose assays employing the Trinder reaction. Of these only 3, ascorbic acid, novaminsulfonic acid and methyl-DOPA were found to give substantial errors when added to the assays in vitro, but had only a negligible effect in assays carried out by in vitro experiments.

Highly icteric or lipemic samples may require a blank correction, using the same volume of sample with isotonic saline in the place of the reagent.

Young et al. (3) published a comprehensive list on the Effect of Drugs on Clinical Laboratory Tests, including glucose assays.

**MATERIALS REQUIRED BUT NOT PROVIDED**
1. Spectrophotometer or colorimeter capable of measuring accurately absorbance at 500 nm.
3. Constant temperature incubator set at 30 °C or 37 °C.
   - Use the same temperature for the assay of standard, controls and samples.
4. Distilled or deionized water.
5. Pipettes to measure distilled or deionized water, reagents and samples.
6. Amber glass bottles for storage of reagent.
7. Standard or calibrator with an established value for glucose concentration.

**MATERIALS PROVIDED**
Glucose reagent, enzymatic in dry powder form.

**TEST PROCEDURE**

<table>
<thead>
<tr>
<th>Test</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>Wave</td>
<td>500 nm</td>
</tr>
<tr>
<td>Test</td>
<td>1.5 mL reagent + 10 μL sample</td>
</tr>
<tr>
<td>Standard</td>
<td>1.5 mL reagent + 10 μL standard</td>
</tr>
<tr>
<td>Blank</td>
<td>1.5 mL reagent</td>
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<tr>
<td>Mix well.</td>
<td></td>
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<tr>
<td>Incubate</td>
<td>10 minutes at 37 °C or 15 minutes at 30 °C</td>
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<tr>
<td>Reading</td>
<td>Set instrument to 0 absorbance with the blank. Read absorbance of the test and standard within 15 minutes from the end of the incubation period recommended above.</td>
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Time of incubation needed to reach completion may be longer, particularly if the reagent was not at the recommended temperature when the sample was added or with reagents reconstituted and stored for prolonged periods under unsuitable storage conditions. Insure that the reaction has reached completion in the time employed.

**CALIBRATION**
This assay requires the use of a glucose standard. Use Multi-Analyte Serum Calibrator (Cat. No. R60010), or other commercially available standards or calibrators.

**CALCULATIONS**

\[
C_a = \text{Glucose value of the standard in mg/dL} \\
A \text{ sample} \times C_a = \text{Glucose in sample in mg/dL}.
\]

**QUALITY CONTROL**
Serum controls are recommended to monitor the performance of manual and automated assay procedures, providing a continued screening of the instrument, reagents and technique. Commercially available control material...
with established values for glucose concentrations may be used. Assayed Control Serum, Level 1 (Cat. No. R83082) and Level 2 (Cat. No. R83083) are recommended for this purpose.

**LIMITATIONS OF THE PROCEDURE**

Samples with glucose concentrations greater than 500 mg/dL should be diluted with an equal volume of distilled or deionized water and the assay repeated; multiply the results by 2.

**REAGENT PERFORMANCE**

Linearity: The assay is linear to 500 mg/dL.

Correlation. Employing as reference Cliniqa Glucose HK (Cat. R80017) in 60 serum samples the correlation coefficient was 0.996 and the regression equation was \( y = 0.95x + 3.7 \).

**REFERENCE RANGE (4)**

70-105 mg/dL

It is recommended that each laboratory establish its own range.

**REFERENCES**