GLUCOSE (GO) ASSAY KIT

Product Description
Enzymes, as analytical tools, have found widespread use in the food, biochemical, and pharmaceutical industry. Enzymatic methods are specific, reproducible, sensitive, rapid, and therefore, ideal for analytical purposes. Due to the high specificity and sensitivity of enzymes, quantitative assays may be done on crude materials with little or no sample preparation. This kit is for the quantitative, enzymatic determination of glucose in food and other materials.

Principle
Glucose is oxidized to gluconic acid and hydrogen peroxide by glucose oxidase. Hydrogen peroxide reacts with o-dianisidine in the presence of peroxidase to form a colored product. The intensity of the pink color measured at 540 nm is proportional to the original glucose concentration.

Reagents
1. Glucose Oxidase/Peroxidase Reagent (Product No. G 3660)
   Store the unopened kit reagent at 2-8 °C. Each capsule contains 500 units of Glucose Oxidase (Aspergillus niger), 100 Purpurogallin units of peroxidase (horseradish) and buffer salts. In an amber bottle, dissolve the contents of the capsule in 39.2 ml of deionized water. The solution is stable up to one month at 2-8 °C and for at least 6 months frozen at −20 °C. Discard if turbidity develops.
2. o-Dianisidine Reagent (Product No. D 2679)
   Store the unopened kit reagent at 2-8 °C. Minimize exposure to light. Preweighed vial contains 5 mg of o-dianisidine dihydrochloride. Reconstitute the vial of o-dianisidine with 1.0 ml of deionized water. Invert the vial several times to dissolve. Avoid exposing the reagent to light. Solution is stable for 3 months at 2-8 °C.
3. Assay Reagent
   Add 0.8 ml of the o-Dianisidine Reagent to the amber bottle containing the 39.2 ml of Glucose Oxidase/Peroxidase Reagent. Invert bottle several times to mix. Minimize exposure to light. Solution is stable up to 1 month at 2-8 °C. Discard if turbidity develops or color forms.
   D-Glucose, 1.0 mg/ml in 0.1% benzoic acid. Store reagent at 2-8 °C. Supplied ready to use. Solution is stable at 2-8 °C for at least six months. Discard if turbidity develops.

Apparatus
1. Spectrophotometer or colorimeter suitable for measuring absorbance at 540 nm.
2. Cuvettes
3. Test tubes, 18 mm X 150 mm
4. Pipettes capable of accurately dispensing volumes from 20 µl to 2.0 ml.
5. Water bath capable of maintaining temperature at 37 ± 1 °C.

Precautions and Disclaimer
Refer to Material Safety Data Sheets for updated risk, hazard, or safety information.

Procedure
Sample Preparation
Liquids:
   Dilute sample with deionized water to approximately 20 - 80 µg glucose/ml. Filter or deproteinize solution if necessary to clarify. Decolorize solutions that are strongly colored and that have a low glucose concentration. Degas carbonated or fermented products.
Solids:
Weigh out sample to nearest 0.1 mg. Extract sample with deionized water. The solution may be heated (<75 °C) to aid extraction. Dilute with deionized water to approximately 20 - 80 µg glucose/ml. Filter or deproteinize solution if necessary to clarify.

Determination

Method 1 - Glucose Concentration from Standard Curve

1. Pipette the following solutions into the appropriately marked test tubes:

<table>
<thead>
<tr>
<th>Tube</th>
<th>Water (ml)</th>
<th>Sample (ml)</th>
<th>Glucose Standard (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent Blank</td>
<td>1.00</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Standard # 1</td>
<td>0.98</td>
<td>---</td>
<td>0.02</td>
</tr>
<tr>
<td>Standard # 2</td>
<td>0.96</td>
<td>---</td>
<td>0.04</td>
</tr>
<tr>
<td>Standard # 3</td>
<td>0.94</td>
<td>---</td>
<td>0.06</td>
</tr>
<tr>
<td>Standard # 4</td>
<td>0.92</td>
<td>---</td>
<td>0.08</td>
</tr>
<tr>
<td>Test</td>
<td>---</td>
<td>1.00</td>
<td>---</td>
</tr>
</tbody>
</table>

2. At zero time, start the reaction by adding 2.0 ml of Assay Reagent to the first tube and mixing. Allow a 30 to 60 second interval between additions of Assay Reagent to each subsequent tube.
3. Let each tube react exactly 30 minutes at 37 °C. Stop reaction at 30-60 second intervals by adding 2.0 ml of 12 N H₂SO₄ into each tube. Carefully mix each tube thoroughly.
4. Measure the absorbance of each tube against the reagent blank at 540 nm.

Calculations:

Method 1

For standards, plot Absorbance at 540 nm (y axis) vs mg of glucose (x axis). If the standard curve is not linear, results will be inaccurate. Repeat assay.

For test, determine mg glucose from standard curve.

Multiply the mg glucose determined above by the dilution factor made in sample preparation.

Method 2

\[
\text{mg Glucose} = \left( \frac{\Delta A_{540} \text{ of Test}}{\Delta A_{540} \text{ of Standard}} \right) \left( \frac{0.05}{\Delta A_{540} \text{ of Standard}} \right)
\]

Multiply the mg glucose determined above by the dilution factor made in sample preparation.

References

2. Official Methods of Analysis of the AOAC, 16th Edition (1995), sections 32.2.05 and 44.7.12.