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ProductInformation

Glucose (GO) Assay Kit

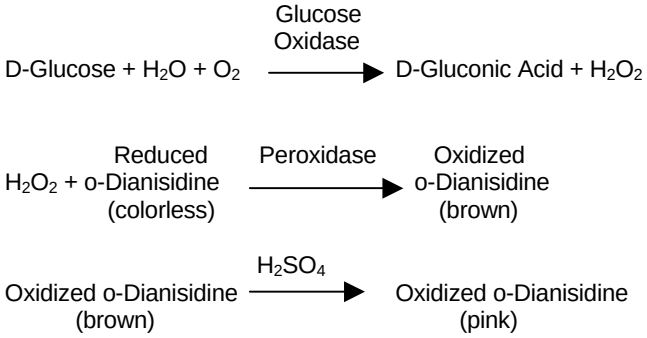
Product Code **GAGO-20**

TECHNICAL BULLETIN

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. Oxidized o-dianisidine reacts with sulfuric acid to form a more stable 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 Code 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. Dissolve the contents of the capsule in an amber bottle with 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 Code D 2679)
   Store the unopened kit reagent at 2–8 °C. Minimize exposure to light. The preweighed vial contains 5 mg of o-dianisidine dihydrochloride. Reconstitute the contents of the o-dianisidine vial 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.
4. Glucose Standard Solution
   (Product Code G 3285)
   D-Glucose, 1.0 mg/ml in 0.1% benzoic acid. This standard is **traceable to an NIST standard** and is supplied ready-to-use. It is stable at 2–8 °C for at least six months. Discard if turbidity develops.

Reagent Required But Not Provided

Sulfuric Acid, ACS reagent (Product Code 25,810-5)
Reagent is 36 N sulfuric acid. Prepare a 12 N solution in deionized water.

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

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

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:

Tube	Water (ml)	Sample (ml)	Glucose Standard (ml)
Reagent Blank	1.00	---	---
Standard # 1	0.98	---	0.02
Standard # 2	0.96	---	0.04
Standard # 3	0.94	---	0.06
Standard # 4	0.92	---	0.08
Test	---	1.00	---

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 the reaction at 30–60 second intervals by adding 2.0 ml of 12 N H_2SO_4 into each tube. Carefully mix each tube thoroughly.
4. Measure the absorbance of each tube against the reagent blank at 540 nm.

Method 2 - Glucose Concentration from a Single Standard

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

Tube	Water (ml)	Sample (ml)	Glucose Standard (ml)
Reagent Blank	1.00	---	---
Standard	0.95	---	0.05
Test	---	1.00	---

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 H_2SO_4 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

$$\text{mg Glucose} = \frac{(\Delta A_{540} \text{ of Test})(\text{mg Glucose in Standard})}{\Delta A_{540} \text{ of Standard}}$$

$$= \frac{(\Delta A_{540} \text{ of Test})(0.05)}{\Delta A_{540} \text{ of Standard}}$$

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

References

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