SIGMA-ALDRICH®
sigma-aldrich.com

3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Glucose (GO) Assay Kit
sufficient for 20 assays

Catalog Number **GAGO20**
Storage Temperature 2–8 °C

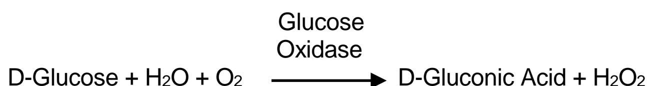
TECHNICAL BULLETIN

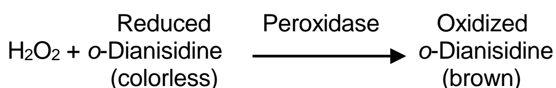
Product Description

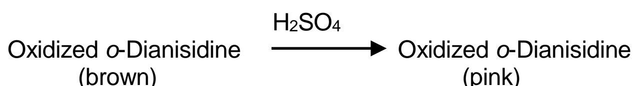
Enzymes, as analytical tools, have found widespread use in the food, biochemical, and pharmaceutical industry. Enzymatic methods are specific, reproducible, sensitive, rapid, and therefore, ideal for analytical purposes. Because of the high specificity and sensitivity of enzymes, quantitative assays may be done on crude materials with little or no sample preparation.[1-5]

This kit is for the quantitative, enzymatic determination of glucose in food and other materials. Various publications have reported use of this kit on different systems and samples, including *Drosophila*,[6-8] dairy manure,[9] cultured mouse muscle cells,[10] HEPG2 cells,[11] and aquatic organisms.[12]

Principle







- Glucose oxidase oxidizes glucose to gluconic acid and hydrogen peroxide.
- 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.

Components

1. Glucose Oxidase/Peroxidase Reagent (Catalog Number G3660)
   - 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.
   - Empty the capsule contents into an amber bottle.
   - Dissolve those contents 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 (Catalog Number D2679)
   - 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 the 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 (Catalog Number G3285)
   - 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 (e.g. Catalog Number 258105): Reagent is 18 M sulfuric acid. Prepare a 6 M solution in deionized water.

Apparatus
1. Spectrophotometer or colorimeter suitable for measuring absorbance at 540 nm.
2. Cuvettes
3. Test tubes, 18 mm × 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
For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Procedure
Sample Preparation
Liquids:
- Dilute sample with deionized water to 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 the nearest 0.1 mg.
- Extract the sample with deionized water. The solution may be heated (<75 °C) to aid extraction.
- Dilute with deionized water to 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 time zero, start the reaction by adding 2.0 mL of Assay Reagent to the first tube and mixing. Allow a 30-60 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 6 M 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 time zero, start the reaction by adding 2.0 mL of Assay Reagent to the first tube and mixing. Allow a 30-60 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 6 M 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.

Results

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, the results will be inaccurate. Repeat the assay.

For the test, determine mg glucose from the standard curve.

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

Calculations, 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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