

EnzyChrom™ Glucose Assay Kit (EBGL-100)

Quantitative Colorimetric/Fluorimetric Glucose Determination

DESCRIPTION

Glucose (C₆H₁₂O₆) is a key diagnostic parameter for many metabolic disorders. Increased glucose levels have been associated with diabetes mellitus, hyperactivity of thyroid, pituitary and adrenal glands. Decreased levels are found in insulin secreting tumors, myxedema, hypopituitarism and hypoadrenalism. Simple, direct and high-throughput assays for measuring glucose concentrations find wide applications in research and drug discovery. BioAssay Systems' glucose assay kit uses a single Working Reagent that combines the glucose oxidase reaction and color reaction in one step. The color intensity of the reaction product at 570nm or fluorescence intensity at λ_{em}/λ_{ex} = 585/530nm is directly proportional to glucose concentration in the sample.

KEY FEATURES

Sensitive and accurate. Use as little as 20 μL samples. Linear detection range in 96-well plate: 5 to 300 μM (90 μg/dL to 5.4 mg/dL) glucose for colorimetric assays and 1 to 30 μM for fluorimetric assays.

Simple and high-throughput. The procedure involves addition of a single working reagent and incubation for 30 min at room temperature.

APPLICATIONS:

Direct Assays: glucose in serum, plasma, urine, saliva, milk, culture medium and other biological samples.

Drug Discovery/Pharmacology: effects of drugs on glucose metabolism.

Food and Beverages: glucose in food, beverages etc.

KIT CONTENTS:

Assay Buffer: 10 mL **Enzyme Mix:** 120 μL

Dye Reagent: 120 μL **Standard:** 1 mL 300 mg/dL Glucose

Storage conditions. The kit is shipped on dry ice. Store Assay Buffer at 4°C and other components at -20°C. Shelf life: 3 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

COLORIMETRIC PROCEDURE

Sample treatment: saliva samples should be centrifuged for 5 min at 14,000 rpm prior to assay. Milk samples should be cleared by mixing 100 μL 6N HCl and 600 μL milk. Centrifuge 5 min at 14,000 rpm and transfer supernatant into a clean tube. Add 170 μL 6N NaOH per mL supernatant. Mix well and centrifuge again at 14,000 rpm. The supernatant can be assayed. The dilution factor in this procedure is *n* = 1.36.

Samples can be analyzed immediately after collection, or stored in aliquots at -20 °C. Avoid repeated freeze-thaw cycles. If particulates are present, centrifuge sample and use clear supernatant for assay.

1. Equilibrate all components to room temperature. During experiment, keep thawed Enzyme in a refrigerator or on ice.

2. **Standards and samples:** for 300 μM standard, mix 15 μL 300 mg/dL standard with 818 μL dH₂O. Dilute standard in dH₂O as follows.

No	300 μM STD + H ₂ O	Vol (μL)	Glucose (μM)
1	200 μL + 0 μL	200	300
2	120 μL + 80 μL	200	180
3	60 μL + 140 μL	200	90
4	0 μL + 200 μL	200	0

Transfer 20 μL standards and samples into separate wells.

3. **Working Reagent.** For each reaction well, mix 85 μL Assay Buffer, 1 μL Enzyme Mix (*vortex briefly before pipetting*), and 1 μL Dye Reagent in a clean tube. Transfer 80 μL Working Reagent into each reaction well. Tap plate to mix.

4. Incubate 30 min at room temperature. Read optical density at 570nm (550-585nm).

CALCULATION

Subtract blank OD (water, #4) from the standard OD values and plot the ΔOD against standard concentrations. Determine the slope and calculate the glucose concentration of Sample,

$$[\text{Glucose}] = \frac{\text{OD}_{\text{SAMPLE}} - \text{OD}_{\text{BLANK}}}{\text{Slope}} \quad (\mu\text{M})$$

OD_{SAMPLE}, OD_{BLANK} are optical density values of the sample and water.

Conversions: 1 mg/dL glucose equals 55.5 μM, 0.001% or 10 ppm.

FLUORIMETRIC PROCEDURE

For fluorimetric assays, the linear detection range is 1 to 30 μM glucose. Mix 10 μL of the standards from *Colorimetric Procedure* with 190 μL dH₂O to obtain standards at 30, 18, 9, 0 μM glucose.

Transfer 20 μL standards and 20 μL samples into separate wells of a black 96-well plate.

Add 80 μL Working Reagent (see *Colorimetric Procedure*), tap plate to mix.

Incubate 30 min at room temperature and read fluorescence at λ_{ex} = 530nm and λ_{em} = 585nm.

The glucose concentration of Sample is calculated as

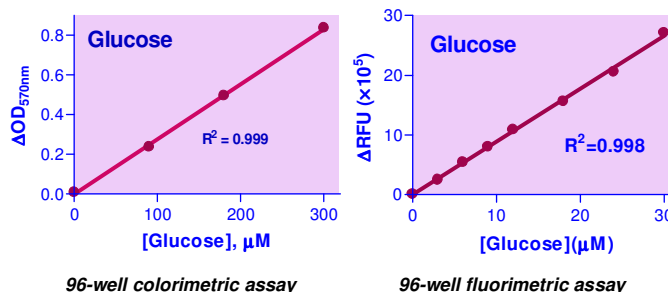
$$[\text{Glucose}] = \frac{F_{\text{SAMPLE}} - F_{\text{BLANK}}}{\text{Slope}} \quad (\mu\text{M})$$

Notes: (1). If the calculated sample glucose concentration is higher than 300 μM in colorimetric assay or 30 μM in fluorimetric assay, dilute sample in water and repeat the assay. Multiply result by the dilution factor. (2). To determine glucose in phenol red culture medium, dilute both sample and glucose standards in the same glucose free medium for colorimetric assay. For fluorimetric assay, prepare standards in phenol red medium. Dilute sample and standards 20-fold or more in water.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, clear flat-bottom 96-well plates, black 96-well plates and plate reader.

Glucose Standard Curves



LITERATURE

- Okuda J, Okuda G. (1969). A rapid polarographic microdetermination of glucose with glucose oxidase. *Clin Chim Acta.* 23(2):365-7.
- Saifer A, Gerstenfeld S. (1958). The photometric microdetermination of blood glucose with glucose oxidase. *J Lab Clin Med.* 51(3):448-60.
- Middleton JE, Griffiths WJ. (1957). Rapid colorimetric micro-method for estimating glucose in blood and C. S. F. using glucose oxidase. *Br Med J.* 2(5060):1525-7.