EnzyChrom™ Glucose Assay Kit (EBGL-100)

Quantitative Colorimetric/Fluorimetric Glucose Determination

DESCRIPTION

Glucose ($C_6H_{12}O_6$) 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 570 nm or fluorescence intensity at $\lambda_{em/ex}$ = 585/530 nm is directly proportional to glucose concentration in the sample.

KEY FEATURES

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

Convenient. Room temperature assay. No 37°C heater is needed.

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, milk, culture medium and other biological samples.
Note: EBGL-100 is not compatible with urine samples. Please consider either EGL2-100 or EGL3-100 for running urine 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 µM	**Standard:**	1 mL (300 mg/dL Glucose)

Storage conditions. The kit is shipped on ice. Store all components at -20°C. Shelf life: 6 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.

COLORIEMTRIC 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 for 5 min. 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 dH_2O. Dilute standard in dH_2O as follows.

No	300 µM STD + H_2O	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 570 nm (550-585 nm).

FLUORIMETRIC PROCEDURE

For fluorimetric assays, the linear detection range is 1 to 30 µM glucose. Mix 20 µL of the standards from *Colorimetric Procedure* with 180 µL dH_2O 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} = 530 nm and λ_{em} = 585 nm.

CALCULATION

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

$$[\text{Glucose}] = \frac{R_{SAMPLE} - R_{BLANK}}{\text{Slope } (\mu M^{-1})} \times n \quad (\mu M)$$

R_{SAMPLE} and R_{BLANK} are optical density or fluorescence intensity readings of the Sample and Blank, respectively. *n* is the sample dilution factor.

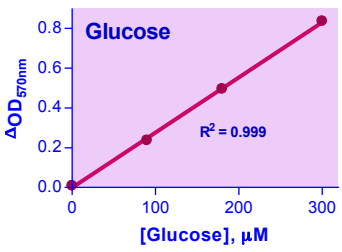
Conversions: 1 mg/dL glucose equals 55.5 µM, 0.001% or 10 ppm.

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.

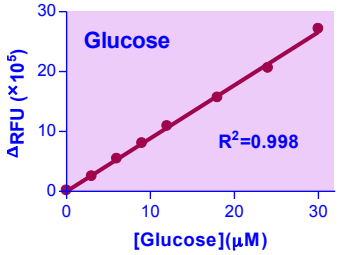
MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, clear flat-bottom 96-well plates (e.g. VWR cat# 82050-760), and plate reader.

Glucose Standard Curves



96-well colorimetric assay



96-well fluorimetric assay

LITERATURE

1. Okuda J, Okuda G. (1969). A rapid polarographic microdeter-mination of glucose with glucose oxidase. Clin Chim Acta. 23(2):365-7.
2. Saifer A, Gerstenfeld S. (1958). The photometric microdeter-mination of blood glucose with glucose oxidase. J Lab Clin Med. 51(3):448-60.
3. 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.