EnzyChrom™ Glucose Assay Kit II (EGL2-100)

Quantitative Colorimetric 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 an enzyme to reduce NAD. The produced NADH, measured at 340 nm, is proportional to the glucose concentration in the sample.

KEY FEATURES

Sensitive and accurate. Use as little as 20 µL samples. Linear detection range in 96-well plate: 0.1 to 3 mM (1.8 mg/dL to 54 mg/dL) glucose for colorimetric assays.

Convenient. The procedure involves adding a single working reagent, and reading the optical density at 20 minutes. 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 20 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	**GDH:**	120 µL
NAD:	1 mL	**Glucose Standard:**	1 mL (300 mg/dL)

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.

SAMPLE PREPARATION

Saliva samples should be centrifuged for 5 min at 14,000 rpm prior to assay. Milk samples should be cleared by mixing 100 µL 6 M HCl and 600 µL milk. Centrifuge 5 min at 14,000 rpm and transfer supernatant into a clean tube. Add 170 µL 6 M 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.

ASSAY PROCEDURE

1. Equilibrate all components to room temperature. During experiment, keep thawed GDH in a refrigerator or on ice.
2. *Standards and samples*: for 3 mM standard, mix 72 µL 300 mg/dL standard with 328 µL dH_2O. Dilute standard in dH_2O as follows.

No	3 mM STD + H_2O	Vol (µL)	Glucose (mM)
1	200 µL + 0 µL	200	3.0
2	120 µL + 80 µL	200	1.8
3	60 µL + 140 µL	200	0.9
4	0 µL + 200 µL	200	0

Transfer 20 µL standards and samples into separate wells.

3. Prepare sufficient Working Reagent (WR) by mixing for each standard and sample well: 80 µL Assay Buffer, 1 µL GDH, and 8 µL NAD in a clean tube. Transfer 80 µL WR into each reaction well. Tap plate briefly and thoroughly to mix.
4. Incubate 20 min at room temperature. Read optical density at 340 nm.

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 as follows:

$$[\text{Glucose}] = \frac{OD_{SAMPLE} - OD_{BLANK}}{\text{Slope}} \times n \quad (\text{mM})$$

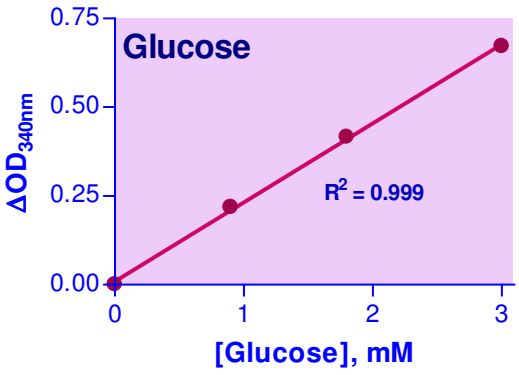
where OD_{SAMPLE}, OD_{BLANK} are optical density values of the sample and water, respectively and n is the sample dilution factor.

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 3 mM, dilute sample in water and repeat the assay. Multiply result by the dilution factor, *n*. (2) To determine glucose in phenol red culture medium, dilute both sample and glucose standards in the same glucose free medium for colorimetric assay.

MATERIALS REQUIRED, BUT NOT PROVIDED

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



Standard Curve in 96-well plate assay in water.

LITERATURE

1. Cho SJ, Cho CH. (2015). Interference Reduction in Glucose Detection by Redox Potential Tuning: New Glucose Meter Development. Anal Sci. 31(7):705-10.
2. Kim DM, Kim MY. (2013). Electron-transfer mediator for a NAD-glucose dehydrogenase-based glucose sensor. Anal Chem. 85(23):11643-9.
3. Saleh FS, Mao L. (2012). A promising dehydrogenase-based bioanode for a glucose biosensor and glucose/O2 biofuel cell. Analyst. 137(9):2233-8.