

## EnzyChrom™ Glucose Assay Kit (EBGL-100)

### Quantitative Colorimetric/Fluorimetric Glucose Determination

#### DESCRIPTION

Glucose (C<sub>6</sub>H<sub>12</sub>O<sub>6</sub>) 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 λ<sub>em/ex</sub> = 585/530 nm 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) 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

<b>Assay Buffer:</b>	10 mL	<b>Enzyme Mix:</b>	120 μL
<b>Dye Reagent:</b>	120 μM	<b>Standard:</b>	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.

#### 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 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<sub>2</sub>O. Dilute standard in dH<sub>2</sub>O as follows.

No	300 μM STD + H <sub>2</sub> 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 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<sub>2</sub>O 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<sub>ex</sub> = 530 nm and λ<sub>em</sub> = 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,

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

R<sub>SAMPLE</sub> and R<sub>BLANK</sub> are optical density or fluorescence intensity readings of the Sample and Blank, respectively. *n* is the sample dilution factor.

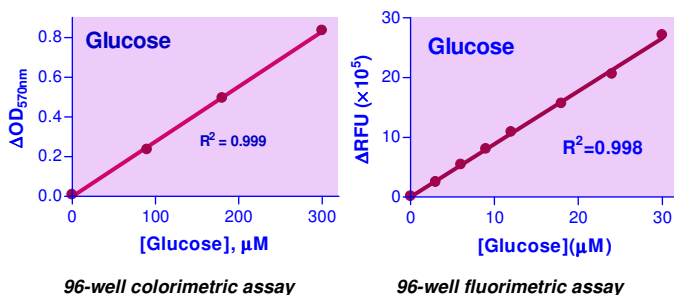
**Conversions:** 1 mg/dL glucose equals 55.5 μM, 0.001% or 10 ppm.

**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



#### PUBLICATIONS

1. Jeong, S., et al (2021). High fructose drives the serine synthesis pathway in acute myeloid leukemic cells. *Cell Metabolism*, 33(1), 145-159.e6.
2. Edriss, H., et al (2020). Advanced glycation end products and glycosaminoglycans in patients with diabetic ketoacidosis. *Journal of Investigative Medicine: The Official Publication of the American Federation for Clinical Research*, 68(3), 738-742.
3. He, L., et al (2014). Potential biomarker of metformin action. *J Endocrinol*. JOE-14-0084.

