

# Glucose Assay Kit II (Colorimetric)

LS-K230-100 (100 Tests) • Store at -20°C



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## Introduction

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. LSBio's 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.

## Components

Component	K230-100
	100 Tests
Assay Buffer	10 mL
NAD	1 mL
GDH	120 µL
Glucose Standard (300 mg/dL)	1 mL

## Materials Not Supplied

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

## Storage

The kit is shipped on ice. Store all kit components at -20 °C.

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**FOR RESEARCH USE ONLY! Not for use in humans.**

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## Assay Procedure

Saliva samples should be centrifuged for 5 min at 14,000 rpm prior to assay. Milk samples should be cleared by mixing 100  $\mu$ L 6 M HCl and 600  $\mu$ L milk. Centrifuge 5 min at 14,000 rpm and transfer supernatant into a clean tube. Add 170  $\mu$ L 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  $-20^{\circ}\text{C}$ . 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 GDH in a refrigerator or on ice.
2. Standards and samples: for 3 mM standard, mix 72  $\mu$ L 300 mg/dL standard with 328  $\mu$ L dH<sub>2</sub>O. Dilute standard in dH<sub>2</sub>O as follows.

No	3 mM STD + H <sub>2</sub> O	Vol ( $\mu$ L)	Glucose (mM)
1	200 $\mu$ L + 0 $\mu$ L	200	3.0
2	120 $\mu$ L + 80 $\mu$ L	200	1.8
3	60 $\mu$ L + 140 $\mu$ L	200	0.9
4	0 $\mu$ L + 200 $\mu$ L	200	0

Transfer 20  $\mu$ L standards and samples into separate wells.

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

## Calculations

Subtract blank OD (water, #4) from the standard OD values and plot the  $\Delta$ OD against standard concentrations. Determine the slope and calculate the glucose concentration of Sample as follows:

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

where  $\text{OD}_{\text{SAMPLE}}$ ,  $\text{OD}_{\text{BLANK}}$  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  $\mu$ M, 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.

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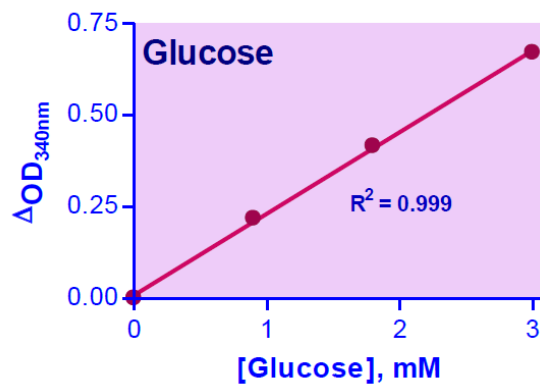
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## Sample Data



Standard Curve in 96-well plate assay in water.

Version: V.08.09.2018

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