Glucose Assay Kit (384-Well) (Colorimetric)

LS-K912-384 (384 Tests) • **See Storage Conditions Below**

Introduction

Glucose ($C_6H_{12}O_6$, FW: 180.16) is delivered to the cells from the blood stream as the primary biological fuel to generate the universal energy molecule, ATP. Even though the human body has evolved an advanced metabolic pathway to keep the blood glucose level in normal range, failure happens and that leads to the development of conditions of persistent high or low blood sugar. Diabetes mellitus is one such most important metabolic disorder where an increased blood sugar level develops compared to normal blood sugar level. Glucose level in blood is also a key diagnostic parameter for many metabolic disorders and its measurement is very important in both research and drug discovery. LSBio's Glucose Colorimetric Assay Kit uses a glucose enzyme mix which oxidizes glucose specifically and produces a product which reacts with a chromophore generating a stable signal (OD: 590 nm). This color is directly proportional to the amount of glucose present in the sample. The method is quantitative, rapid, simple, sensitive, and designed to be used in high-throughput settings. The kit can detect 0.5 to 5 mM of glucose in various biological samples.

Applications

- Measurement of Glucose in various biological samples
- Growth media
- Analysis of carbohydrate metabolism
- Analysis of glucose content in foodstuff

Sample Types

- Serum, plasma, & other body fluids
- Growth media
- Bacteria, yeast cultured samples
- Milk, juice, etc.

Components

Component	K912-384 384 Tests	Cap Code
Glucose Assay Buffer	25 ml	WM
Glucose Probe	0.8 ml	Red
Glucose Enzyme Mix (Lyophilized)	1 vial	Green
Glucose Standard (100 mM)	100 µl	Yellow

Materials Not Supplied

- 384-well clear plate with flat bottom
- 10kDa Spin Column

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LSBio
LifeSpan BioSciences, Inc.

Storage Conditions and Reagents Preparation

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- Glucose Assay Buffer: Warm to room temperature prior to use. Store at -20°C or 4°C.
- Glucose Probe: Ready to use as supplied. Warm to room temperature prior to use to thaw contents. Store at -20°C, protected from light and moisture. Use within two months. Aliquot to avoid multiple freeze/thaw cycles.
- Glucose Enzyme Mix: Dissolve in 220 µl Glucose Assay Buffer. Aliquot & store at -20°C. Keep on ice while in use. Use within two months.

Assay Procedure

1. Sample Preparation: Add 1.0 to 12.5 µl of sample directly to a 384-well clear flat bottom plate. Adjust the volume to 12.5 µl/well with Glucose Assay Buffer. For serum, limit sample volume to 1 µl or dilute serum. Serum in healthy patients contains < 6.0 nmol/µl glucose. Adjust the final volume to 12.5 µl with Glucose Assay Buffer.

   Notes:

   a) For unknown samples, we suggest performing a pilot experiment & testing different sample dilutions with the Assay Buffer to ensure the readings are within the Standard Curve range. Though this kit has been optimized using serum from a healthy donor by adding sample directly to the well, pilot experiments are strongly encouraged to be carried out if samples are suspected to have abnormal Glucose concentrations.

   b) For sample having background, prepare parallel background well(s) containing same amount of sample as in the test well.

   c) Endogenous enzyme activity may cause loss of glucose. Samples should be deproteinized using a 10kDa Spin Column.

   d) Instrument reader settings need to be adjusted according to the chosen 384-well plate. (The right dimension of the used 384-well plate may be available in the manual provided by the plate manufacturer).

2. Glucose Standard Curve: Dilute the Glucose Standard to 0.5 mM by adding 5 µl of the Glucose Standard to 995 µl of Glucose Assay Buffer, mix well. Add 0, 2, 4, 6, 8, 10 µl into a series of wells on a 384-well plate. Adjust volume to 12.5 µl/well with Glucose Assay Buffer to generate 0, 1, 2, 3, 4, 5 nmol/well of Glucose Standard.

3. Glucose Reaction Mix: Mix enough reagents for the number of assays to be performed. For each well, prepare 12.5 µl Mix containing:

	Reaction Mix	*Background Control Mix
Glucose Assay Buffer	10.0 µl	10.5 µl
Glucose Probe	2.0 µl	2.0 µl
Glucose Enzyme Mix	0.5 µl	----

   Mix and add 12.5 µl of the Reaction Mix to each well containing the Standard, and test samples. Mix well.

   *For samples having background, add 12.5 µl of the background control mix to sample background control well(s).

4. Measurement: Incubate the reaction for 30 min. at 37°C, protected from light. Measure absorbance (OD 590 nm) in a microplate reader.

5. Calculation: Subtract 0 Standard reading from all readings which will be the corrected absorbance readings. If the sample background control reading is significant then subtract the sample background control reading from sample reading. Plot the Glucose Standard Curve. Apply the corrected absorbance of the sample to the Glucose Standard Curve to get B nmol of Glucose in the sample well.

   Sample Glucose concentration (C) = B/V X D nmol/µl or mM

   Where: B = is the amount of Glucose in the sample well from Standard Curve, V = sample volume added into the reaction well (µl), D = Dilution Factor.

   Glucose molecular weight: 180.2 g/mol.

   1 mM ≡ 18.08 mg/dl.

Sample Data

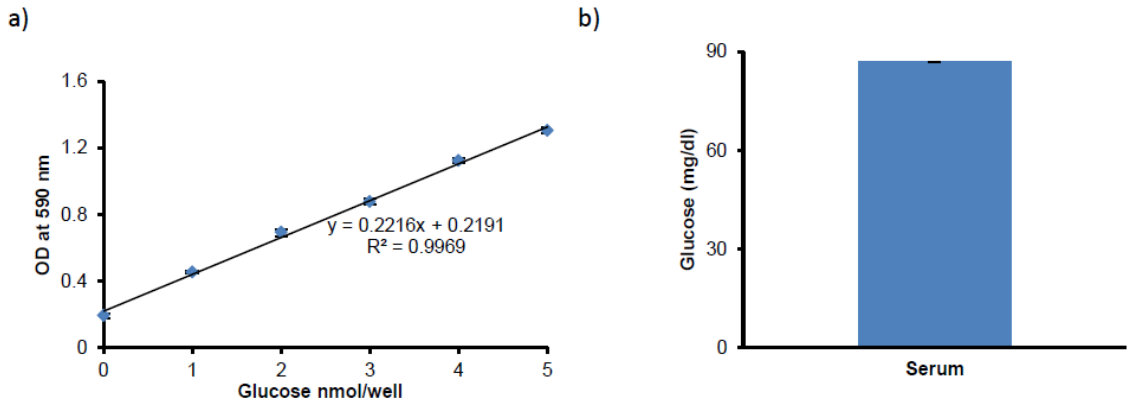

Figure: (a) Glucose Standard Curve. (b) Quantitation of Glucose in human serum. Serum samples were deproteinized using a 10kDa Spin Column (10,000 x g, 10 min, 4°C). Undiluted serum filtrate (1 µl) samples were added to the wells directly. Assays were performed according to the kit protocol. Calculated concentration: 86.78 ± 0.1 mg/dl.

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