Glucose Assay Kit I

High-throughput adaptable colorimetric & fluorometric assay to determine glucose in various biological samples

PromoKine

Instruction Manual

Catalog Number	PK-CA577-K606
Description	Glucose ($C_6H_{12}O_6$, FW: 180.16) is the primary biological fuel source used to generate the universal energy molecule, ATP. Glucose level is a key diagnostic parameter for many metabolic disorders and its measurement is very important in both research and drug discovery processes. PromoKine's Glucose Assay Kit I provides direct measurement of glucose in various biological samples (see below). Glucose Enzyme Mix oxidizes glucose specifically, to generate a product which reacts with a dye to generate color (OD 570 nm) and fluorescence (Ex/Em = 535/587 nm). The generated color or fluorescence is directly proportional to the amount of glucose present. The method is rapid, simple, sensitive, and suitable for high throughput. The assay is also suitable for monitoring glucose levels during fermentation and glucose feeding in protein expression processes. The kit detects 1-10,000 µM of glucose in various samples.
Quantity	100 assays
Applications	• Measurement of glucose in various samples • Analysis of carbohydrate metabolism
Sample Type	• Serum, plasma, urine & other body fluids • Growth media • Food

Components	Quantity	Color Code
Glucose Assay Buffer	25 ml	WM
Glucose Probe (in DMSO)	200 µl	Red
Glucose Standard (100 nmol/µl)	100 µl	Yellow
Glucose Enzyme Mix (lyophilized)	1 vial	Green

User Supplied Reagents & Equipment	• 96-well plate with flat bottom • Multi-well spectrophotometer
Storage and Reagents Preparation	Store kit at –20°C, protected from light. Briefly centrifuge small vials prior to opening. Read the 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 melt frozen DMSO. Store at -20°C, protect from light and moisture. Use within two months. • **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 Protocol	**1. Sample Preparation**: Add 2-50 µl test samples to a 96-well plate. Adjust the volume to 50 µl/well with Glucose Assay Buffer. If using serum, limit sample volume to 0.5-2 µl/assay. Normal serum contains ~5 nmol/µl glucose. Urine can be assayed directly. Adjust the final volume to 50 µl with Assay buffer. Note: 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. b. For samples having background, prepare parallel well(s) containing same amount of sample as in the test well. c. Endogenous compounds may interfere with the reaction. To ensure accurate determination of glucose in the test samples, we recommend spiking samples with a known amount of Standard (4 nmol). d. Endogenous enzyme activity may cause loss of glucose. All samples containing enzyme activity should be deproteinized using a 10 kDa Spin Column (Cat.No. PK-CA577-1997). **2. Standard Curve Preparation**: For colorimetric assay, dilute the Glucose Standard to 1 nmol/µl by adding 10 µl of the Glucose Standard to 990 µl of Glucose Assay Buffer, mix well. Add 0, 2, 4, 6, 8, 10 µl into a series of wells on a 96 well plate. Adjust volume to 50 µl/well with Glucose Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of Glucose Standard. For the fluorometric

assay, dilute the Glucose Standard solution to 0.1 nmol/µl by adding 10 µl of the Glucose Standard to 990 µl of Glucose Assay Buffer, mix well. Then take 20 µl into180 µl of Glucose Assay Buffer. Mix well. Add 0, 2, 4, 6, 8, 10 µl into a series of wells as in the colorimetric assay. Adjust volume to 50 µl/well with Glucose Assay Buffer to generate 0, 0.2, 0.4, 0.6, 0.8, 1.0 nmol/well of the Glucose Standard.

3. Glucose Reaction Mix: Mix enough reagent for the number of assays to be performed: For each well, prepare a total 50 µl Reaction Mix containing:

	Reaction Mix	**Background Control Mix***
Glucose Assay Buffer	46 µl	48 µl
Glucose Probe**	2 µl	2 µl
Glucose Enzyme Mix	2 µl	-----

Mix well. Add 50 µl of the Reaction Mix to each well containing the Glucose Standard and test samples. Mix well. Note:

* For samples having background, add 50 µl of the background control mix to sample background control well(s)

** The fluorometric assay is ~10 times more sensitive than the colorimetric assay. Use 0.4 µl of the probe per reaction to decrease background/increase detection sensitivity significantly.

4. Measurement: Incubate the reaction for 30 minutes at 37°C, protect from light. Measure absorbance (OD 570 nm) or Fluorescence (Ex/Em = 535/590 nm) for in a microplate reader.

5. Calculations: Subtract 0 Standard reading from all readings. If sample background control reading is significant then subtract the sample background control reading from sample reading. Plot the Glucose Standard Curve. For unspiked samples, apply the corrected absorbance or fluorescence 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 (nmol)

V is the sample volume added into the reaction well (µl)

D is the sample dilution factor

Note: For spiked samples, correct for any sample interference by subtracting the sample reading from spiked sample reading. Glucose molecular weight: 180.2 g/mol.

For spiked samples, Glucose amount in sample well (B) =

$$\left(\frac{(OD_{\text{sample (corrected)}})}{(OD_{\text{sample + Glucose Std(corrected)}})-(OD_{\text{sample(corrected)}})}\right) * Glucose\ Spike\ (\text{nmol})$$

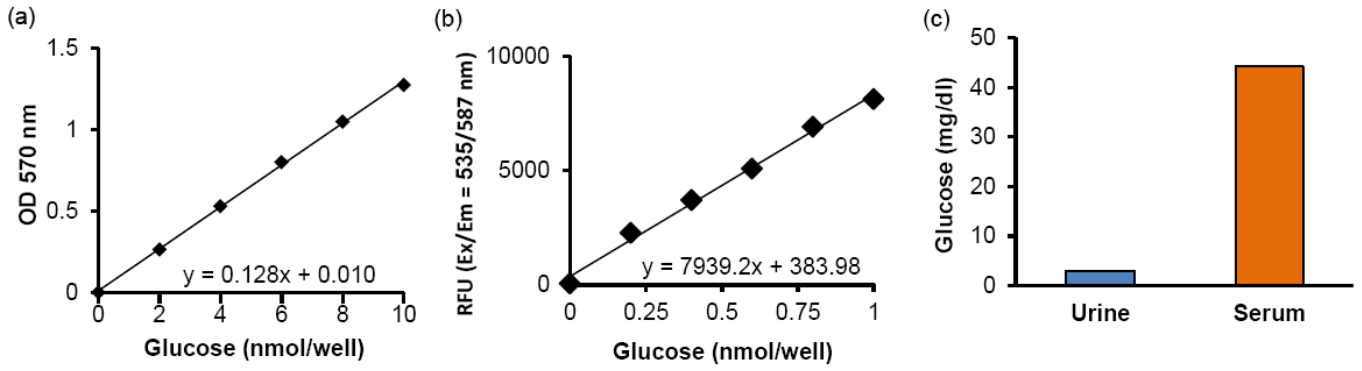

Figure: Glucose Standard Curve; (a) Colorimetric (b) Fluorometric, (c) Quantitation of Glucose in human urine & serum. Urine & serum samples were deproteinized using a 10 kDa Spin Column (10000xg, 10 minutes, 4°C). Urine filtrate (20 µl) & serum filtrate (1 µl) were spiked with a known amount of glucose as internal standard (4 nmol). Assays were performed according to the kit protocol. Calculated concentrations: Urine: 3.00 ± 0.4 mg/dl; Serum: 44.2 ± 6.7 mg/dl.

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