QuantiChrom™ Glucose Assay Kit (DIGL-200)

Quantitative Colorimetric Glucose Determination at 630nm

DESCRIPTION

Glucose ($C_6H_{12}O_6$) is a ubiquitous fuel molecule in biology. It is oxidized through a series of enzyme-catalyzed reactions to form carbon dioxide and water, yielding the universal energy molecule ATP. Due to its importance in metabolism, glucose level 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 automation-ready procedures for measuring glucose concentrations find wide applications in research and drug discovery. BioAssay Systems' glucose assay kit is designed to measure glucose directly in serum or plasma without any pretreatment. The improved *o*-toluidine method utilizes a specific color reaction with glucose. The absorbance at 630nm is directly proportional to glucose concentration in the sample.

KEY FEATURES

Sensitive and accurate. Use as little as 5 µL samples. Linear detection range 0.7 mg/dL (39 µM) to 300 mg/dL (16.6 mM) glucose in 96-well plate.
Simple and convenient. The procedure involves addition of a single working reagent and incubation for 8 min in a boiling water bath.
Improved reagent stability. The optimized formulation has greatly enhanced the reagent and signal stability.
Low interference in biological samples. No pretreatments are needed. Assays can be directly performed on serum and plasma samples.

APPLICATIONS:

Direct Assays: glucose in biological samples (e.g. serum and plasma).
Drug Discovery/Pharmacology: effects of drugs on glucose metabolism.
Food and Beverages: glucose in food, beverages etc.

KIT CONTENTS (200 tests in 96-well plates)

Reagent: 100 mL Standard: 1 mL 300 mg/dL glucose

Storage conditions. The reagent and standard should be stored at room temperature and -20°C, respectively. Shelf life: 12 months.

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.

PROCEDURES

IMPORTANT: THE REAGENT CONTAINS ACETIC ACID. THIS ASSAY IS PREFERABLY CARRIED OUT IN A CHEMICAL FUME HOOD.

Procedure using 96-well plate:

1. Dilute standard in distilled water as follows.

No	STD + H_2O	Vol (µL)	Glucose (mg/dL)
1	150µL + 0µL	150	300
2	100µL + 50µL	150	200
3	50µL + 100µL	150	100
4	25µL + 125µL	150	50
5	0µL + 150µL	150	0

Set up 1.5-mL centrifuge tubes. Transfer 5 µL diluted standards and samples to appropriately labeled tubes. Transfer 500 µL Reagent to each tube. Close the tubes tightly and mix. Store diluted standards at -20°C for future use.

2. Place the tubes in a tube holder and heat in a boiling water bath for 8 min. Cool down in cold water bath for 4 min.
3. Transfer 200 µL in duplicate into a clear bottom 96-well plate. Careful: avoid bubble formation. Read optical density at 620-650nm (peak absorbance at 630nm). Signal is stable for 60 min.

Procedure using cuvette:

1. Dilute standards and transfer 12 µL Standards and samples to appropriately labeled tubes. Transfer 1200 µL Reagent to each tube. Close the tubes tightly and mix.
2. Place the tubes in a tube holder and heat in a boiling water bath for 8 min. Cool down in cold water bath for 4 min.
3. Transfer 1000 µL into a clear bottom 96-well plate. Read optical density at 620-650nm (peak absorbance at 630nm). Signal is stable for 60 min.

Note: if the Sample OD is higher than the Standard OD at 300mg/dL, dilute the sample in double distilled water and repeat the assay.

CALCULATION

Subtract blank OD (water, #5) from the standard OD values and plot the OD against standard concentrations. Determine the slope using linear regression fitting. The glucose concentration of Sample is calculated as

$$= \frac{OD_{SAMPLE} - OD_{BLANK}}{Slope} \quad (mg/dL)$$

OD_{SAMPLE} and OD_{BLANK} are optical density values of the sample and sample "Blank" (water or buffer in which the sample was diluted). Typical serum/plasma glucose values: 70 - 110 mg/dL.
Conversions: 1mg/dL glucose equals 55.5 µM, 0.001% or 10 ppm.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipeting devices, centrifuge tubes, boiling water bath, tube holder.

Procedure using 96-well plate:

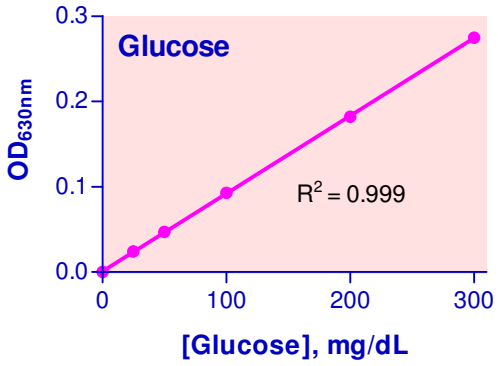
Clear bottom 96-well plates (e.g. Corning Costar) and plate reader.

Procedure using cuvette:

Spectrophotometer and Cuvets for measuring OD at 620-650nm.

EXAMPLES:

Rat plasma, rat serum, goat serum and human plasma were assayed using the 96-well plate assay protocol. The glucose concentrations were 128 ± 2 (n = 4), 72.5 ± 0.8 (n = 4), 78.6 ± 0.6, 69.3 ± 0.7 mg/dL (n = 4), respectively. Coefficient of variance < 3%.



Calibration curve in 96-well plate

LITERATURE

1. Sacks DA, Greenspoon JS, Fotheringham N (1992). Could the fasting plasma glucose assay be used to screen for gestational diabetes? J Reprod Med. 37:907-909.

2. Northam BE, Smith JH, Fitzgerald MG, Nattrass M, Wright AD (1982). Value of serum glucose assay as part of the biochemical profile in screening for diabetes. Ann Clin Biochem. 19:412-415.

3. Giampietro O, Pilo A, Buzzigoli G, Boni C, Navalesi R (1982). Four methods for glucose assay compared for various glucose concentrations and under different clinical conditions. Clin Chem. 28:2405-2407.