Glucose Assay (GL)

Cat. No. 8418
100 Tests in 96-well plate

Introduction

Glucose is the primary source of energy for cells. Glucose level is a key diagnostic parameter for many metabolic disorders such as diabetes, tumors and Alzheimer's disease. Additionally, measurement of glucose can be very important in both diagnostic and research processes. This colorimetric assay is based on glucose oxidase catalyzed oxidation of glucose, in which the formed hydrogen peroxide is catalyzed by peroxidase and reacts with 4-aminoantipyrine to form the product dye. The color intensity of the reaction product at 570nm is directly proportional to glucose concentration in the sample.

Kit Components

Cat. No.	# of vials	Reagent	Quantity	Storage
8418a	1	Assay buffer	10 mL	4°C
8418b	1	Glucose standard	1 mL	-20°C
8418c	1	Substrate mix	1.6 mL	-20°C
8418d	1	Cofactor mix	0.4 mL	-20°C
8418e	1	Enzyme mix	0.2 mL	-20°C

Product Use

Glucose Assay kit could measure glucose level of many types of samples, including serum, plasma, urine and saliva. This product is for research purposes only and not for use in animals, humans, or diagnostic procedures.

Quality Control

Data from Glucose Assay of glucose solutions with concentrations ranging from 0.02 to 1 mg/mL show a linear relationship between OD_{570nm} and glucose concentration (Figure 1).

Shipping

Shipped on dry ice.

Procedure (96-well plate)

A. Preparation of glucose standard

1. Add 10 µL of glucose standard (8418b) to 40 µL of assay buffer (8418a) to make a 0.05 mL solution of 2 mg/mL glucose.
2. Obtain 7 test tubes, add 25 µL of assay buffer (8418a) into each tube and label them #1 through #7.
3. Add 25 µL 2 mg/mL glucose into tube #1 and mix well to get the 1 mg/mL glucose standard.
4. Transfer 25 µL of the 1 mg/mL glucose standard from tube #1 to tube #2 and mix well to get the 0.5 mg/mL glucose standard.
5. Repeat step 4 for tubes #3-6 to serially dilute the glucose standards. Do not add any glucose to tube #7, which serves as blank.
6. Obtain a 96-well test plate, prepare 2 replicates (A, B) of each glucose standard by aliquoting 10 µL/well of each glucose standard into duplicate wells of the 96-well test plate, according to the following plate format:

	#1	#2	#3	#4	#5	#6	#7
A	1 mg/mL	0.5 mg/mL	0.25 mg/mL	0.125 mg/mL	0.0625 mg/mL	0.03125 mg/mL	Blank
B	1 mg/mL	0.5 mg/mL	0.25 mg/mL	0.125 mg/mL	0.0625 mg/mL	0.03125 mg/mL	Blank

B. Preparation of test samples

Samples should be serial diluted to make sure the readings are within the standard curve range. Prepare test samples to a final volume of 10 µL/well on the 96-well flat bottom plate.

C. Working reagent preparation and measurements

1. For each well of reaction, prepare working reagent by mixing 68 µL assay buffer (8418a), 16 µL substrate mix (8418c), 4 µL cofactor mix (8418d) and 2 µL enzyme mix (8418e).
2. Add 90 µL of working reagent mix into each well of the 96-well plate containing glucose standard, samples and blank. Incubate for 30 minutes at room temperature in dark.
3. Read the absorbance at 570 nm with an ELISA plate reader.

D. Calculations

1. Subtract the OD_{570nm} value of the blank from the OD_{570nm} values obtained with all other standard and samples to get ΔOD_{570nm} value.
2. Based on the calibrated ΔOD_{570nm} of the glucose standard, make a standard curve by plotting ΔOD_{570nm} as a function of glucose concentration (See Figure 1 for a typical standard curve). Determine the equation and R^2 value of the trend line.
3. Suppose the equation of the trend line of the standard curve is $y = Ax + B$, calculate the glucose concentration of test samples as follows:

$$[\text{Glucose}] = \frac{\Delta\,\text{OD570nm} - B}{A}$$

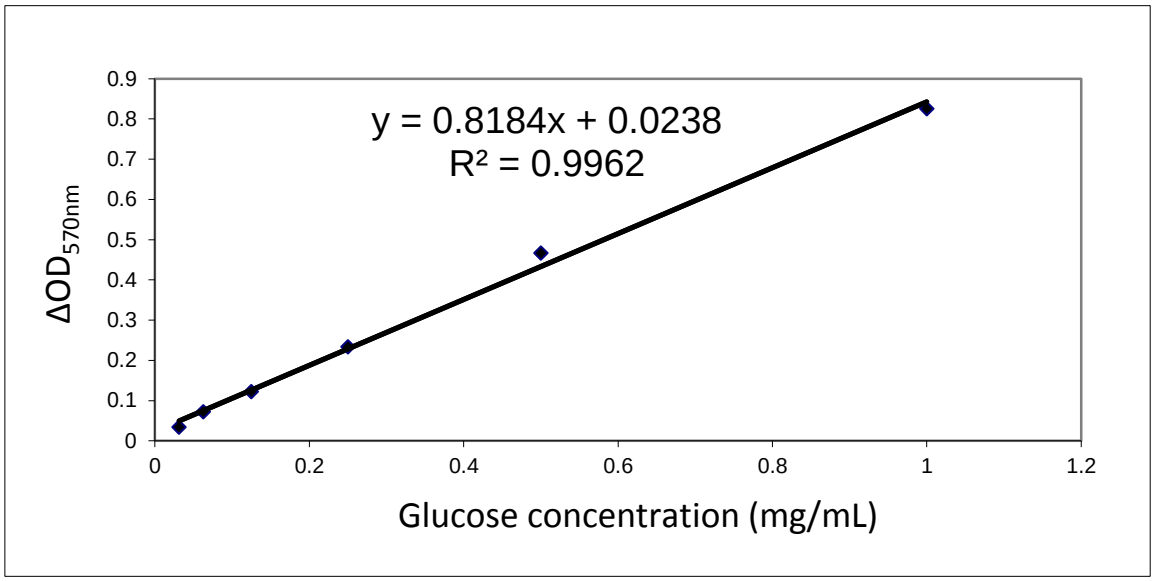

Figure1. A typical glucose standard curve measured by ScienCell™ Glucose Assay kit