MA-0122

Glucose Assay

(100 wells, Colorimetric, OD 570 nm or Fluorometric, Ex/Em = 535/587 nm, Store at -20°C)

Background Information:

Glucose is the primary source of energy in all organisms. In plants, glucose is stored as starch, in animals, glycogen. Blood glucose concentration is highly regulated, and a variety of metabolic disorders is reflected in a significant deviation in glucose concentration. Much of intermediary metabolism is involved in either the synthesis or utilization of glucose to supply energy to the organism in the form of ATP. AkrivisBio's Glucose Assay provides a simple, sensitive measurement of glucose in a wide range of biological samples. In the assay glucose is oxidized, with the formation of hydrogen peroxide which is utilized by peroxidase to convert ADHP to resorufin with the development of intense color and fluorescence.

Assay Principle:

1 – Glucose is oxidized by glucose oxidase, forming gluconolactone and hydrogen peroxide
2 – Peroxidase uses hydrogen peroxide to convert ADHP to resorufin giving absorbance (570 nm) and fluorescence (Excitation: 535 nm, Emission: 587 nm)

Assay Components:

Assay Buffer	25 ml	WM	MA-0122-A
ADHP Solution	0.2 ml	Red	MA-0122-B
Glucose Oxidase/Peroxidase	lyoph	Green	MA-0122-C
Glucose Standard (40 mM)	100 µl	Yellow	MA-0122-D

Storage and Handling:

Store kit at -20°C. Centrifuge all small vials briefly before opening. Bring all components to room temperature before using.
Assay Buffer, ADHP Solution, Glucose Standard: Ready to use as supplied. Store at 4 °C.
Glucose Oxidase/Peroxidase: Reconstitute with 220 µl Assay Buffer. Dissolve and then place on ice. If you plan to use the assay several times over a period of time, aliquot the enzyme solutions into convenient portions and freeze to prevent multiple freeze/thaw cycles which accelerates enzyme denaturation. Store at -20°C after reconstitution.

Assay Protocol:

1. Standard Curve:

2. Absorbance based assay: Dilute the Glucose Standard to 0.4 mM by adding 10 µl of the Glucose Standard to 990 µl of Assay Buffer, mix well. Add 0 – 5 – 10 – 15 – 20 - 25 µl into a series of wells on a 96 well plate. Adjust all well volumes to 50 µl with Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of Glucose Standard.

Fluorescence based assay: Dilute the Glucose Standard solution as in the absorbance-based assay then dilute another 10X to 40 µM by transferring 10 µl of the diluted standard to 90 µl of Assay Buffer. Transfer 0 – 5 – 10 – 15 – 20 – 25 µl to a series of wells on a 96 well plate to give 0, 200, 400, 600, 800, 1000 pmol/well of the Glucose Standard. Adjust all volumes to 50 µl/well with Glucose Assay Buffer.

3. Sample Preparation: Transfer 2-50 µl of unknown samples to wells of a 96-well plate. Dilute serum 10X and use 5-20 µl. Adjust all well volumes to 50 µl with Assay Buffer.
Note: Some samples will give a high background. In that event, rerun the sample including a paired background control.

4. 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

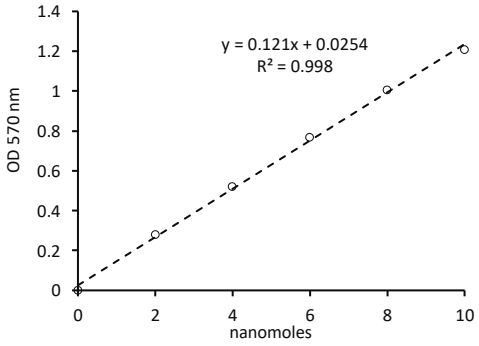
	Reaction Mix	Background Control Mix
Assay Buffer	46 µl	48 µl
Glucose Probe	2 µl	2 µl
Glucose Oxidase/Peroxidase	2 µl	----

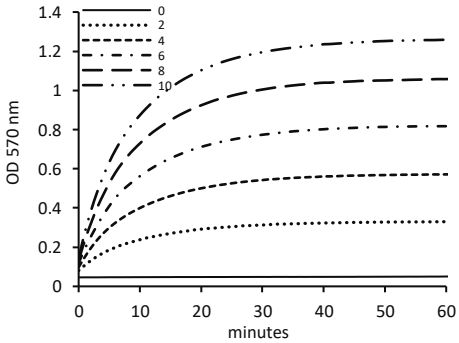
Mix well. Add 50 µl of the Reaction Mix to each well containing the Glucose Standard and test samples. Mix well.

5. Measurement: Monitor the reaction progress with either absorbance (OD 570 nm) or fluorescence (Excitation/Emission = 535/587 nm). When the signal from the standards is no longer changing, the reaction is finished. It should take less than 60 minutes to reach endpoint.

6. Typical Results:

nmole Standard	Standard Raw Values OD	Background Corrected Values
0	**0.0504**	**0**
2	**0.3295**	**0.2791**
4	**0.5716**	**0.5212**
6	**0.8174**	**0.7670**
8	**1.0577**	**1.0073**
10	**1.2586**	**1.2082**





7. Calculations: Subtract 0 Standard reading from all standard readings. Plot the Glucose Standard Curve. Determine the slope of the standard curve. This slope will be used to determine all unknown glucose concentrations. Subtract sample background control reading from sample reading. If there isn't any background control, subtract the 0 standard reading from each unknown sample. Divide the background corrected sample readings by the slope of the standard curve to get the nmoles of glucose in the sample wells. In order to convert to the amount of glucose in the original samples:

A. nmoles per well / µl sample added to well = nmoles per µl of sample
B. nmoles per µl of sample X total µl of sample = total nmoles glucose per sample
C. Total nmoles glucose per sample / mg tissue (or # of cells or µl liquid) of original sample = nmoles/mg (# cells, µl, etc.)

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