KAMIYA BIOMEDICAL COMPANY

Glucose Colorimetric Detection Kit

For the quantitative determination of glucose in serum, plasma, urine, buffers and TCM

Cat. No. KT-725

For Research Use Only.

PRODUCT INFORMATION

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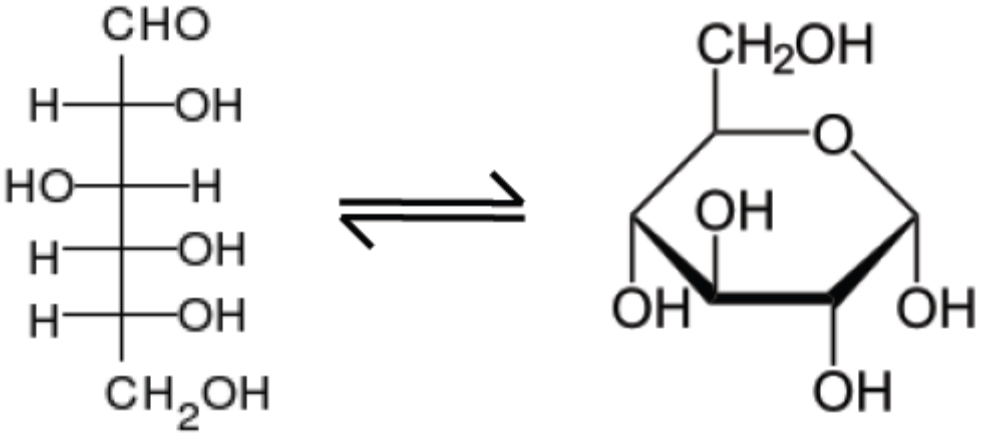
BACKGROUND

Glucose ($C_6H_{12}O_6$) is by far the most common carbohydrate. It is a monosaccharide, an aldose, a hexose, and a reducing sugar and is also known as dextrose, because it is dextrorotatory (rotates polarized light clockwise). The structure of glucose is shown below as both the straight chain and cyclic forms.



Glucose Structures

For all biological and molecular events and for multiple cellular functions, energy is essential. Energy is available in the form of ATP (adenosine triphosphate), most of which is generated through aerobic cellular respiration of carbohydrate and glucose, the major source of biological free energy in higher organisms. Reduced energy levels threaten cellular homeostasis and integrity. Impaired energy metabolism may trigger pro-apoptotic signaling (programmed cell death), oxidative damage, excitotoxicity and impede mitochondrial DNA repair.

A serious fall in blood glucose can be characterized by metabolic dysfunction, neuroglycopenia, seizure, and death. A persistent elevation in blood glucose leads to “glucose toxicity.” Glucose toxicity contributes to ß-cell dysfunction and the pathology grouped together as complications of diabetes. Estrogen-induced signaling pathways in hippocampal and cortical neurons involve the mitochondria to enhance mitochondrial function and to sustain aerobic glycolysis and citric acid cycle oxidative phosphorylation and ATP generation.

PRINCIPLE

The Glucose Colorimetric Detection Kit is designed to quantitatively measure glucose in a variety of samples. Please read the complete kit insert before performing this assay. A ß-D-glucose calibrator is provided to generate a calibration curve for the assay and all samples should be read off the calibration curve. Samples are mixed with the Colorimetric Substrate and horseradish peroxidase and the reaction initiated by addition of glucose oxidase. The reaction is incubated at room temperature for 30 minutes. The glucose oxidase reacts with glucose to produce hydrogen peroxide which, in the presence of HRP, reacts with the Colorimetric Substrate to convert the colorless substrate into a pink-colored product. The pink

product is read at 560 nm. Increasing levels of glucose cause a linear increase in color.

COMPONENTS

Clear 96 well Half Area Plates 2 Plates
Corning Costar Plate 3695.

Glucose Calibrator 90 µL
Glucose at 320 mg/dL in a special stabilizing solution.

Assay Buffer 50 mL
Assay buffer containing detergents and stabilizers.

Colorimetric Substrate 5 mL
A solution of the substrate in a special stabilizing buffer.

Horseradish Peroxidase Concentrate 60 µL
A 100X concentrated solution of HRP in a special stabilizing solution.

Glucose Oxidase Concentrate 600 µL
A 10X concentrated solution of Glucose Oxidase in a special stabilizing solution.

STORAGE

All components of this kit should be stored at 4℃ until the expiration date of the kit.

OTHER MATERIALS REQUIRED

Repeater pipet with disposable tips capable of dispensing 25 µL.

96 well plate reader capable of reading at 560 nm (Acceptable Range 540-580 nm.). Set plate parameters for a 96-well Corning Costar 3695 plate.

Software for converting colorimetric intensity readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting. Contact your plate reader manufacturer for details.

PRECAUTIONS

As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product. **This product is not for Human Diagnostic Use.**

SAMPLE TYPES AND PREPARATION

Samples that need to be stored after collection should be stored at -70℃ or lower, preferably after being frozen in liquid nitrogen. Serum and plasma samples can be used after being diluted ≥ 1:15. This assay has been validated for buffer and media samples.

REAGENT PREPARATION

Horseradish Peroxidase (HRP) and Glucose Oxidase (GOD) Preparation
Dilute the HRP Stock solution 1:100 with Assay Buffer using the table below:

HRP Dilution Table

	1/2 Plate	One Plate	Two Plates
HRP Stock	15 µL	30 µL	55 µL
Assay Buffer	1.485 mL	2.97 mL	5.445 mL
Total Volume	1.5 mL	3 mL	5.5 mL

Dilute the GOD Stock solution 1:10 with Assay Buffer using the table below:

GOD Dilution Table

	1/2 Plate	One Plate	Two Plates
GOD Stock	150 µL	275 µL	550 µL
Assay Buffer	1.350 mL	2.475 mL	4.95 mL
Total Volume	1.5 mL	2.75 mL	5.5 mL

Calibrator Preparation
Glucose Calibrators are prepared by labeling seven tubes as #1 through #7. Briefly vortex to mix the vial of Glucose Calibrator. Pipet 135 µL of Assay Buffer into tube #1. Pipet 75 µL of Assay Buffer into tubes #2 to #7. Carefully add 15 µL of the Glucose Calibrator to tube #1 and vortex completely. Take 75 µL of the solution in tube #1 and add it to tube #2 and vortex completely. Repeat this for tubes #3 through #7. The concentration of glucose in tubes 1 through 7 will be 32, 16, 8, 4, 2, 1, and 0.5 mg/dL.

Use all Calibrators within 2 hours of preparation.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7
Assay Buffer (µL)	135	75	75	75	75	75	75
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 5
Vol of Addition (µL)	15	75	75	75	75	75	75
Final Conc (mg/dL)	32	16	8	4	2	1	0.5

ASSAY PROTOCOL

Use the plate layout sheet on the back page to aid in proper sample and calibrator identification. Set plate parameters for a 96-well Corning Costar 3695 plate.

1. Pipet 20 µL of diluted samples or calibrators into duplicate wells in the plate.

2. Pipet 20 µL of Assay Buffer into duplicate wells as the Zero calibrator.

3. Add 25 µL of the prepared HRP solution to each well using a repeater pipet.

4. Add 25 µL of the Colorimetric Substrate solution to each well using a repeater pipet.

5. Initiate the reaction by adding 25 µL of the prepared GOD solution to each well using a repeater pipet.

6. Incubate at room temperature for 30 minutes.

7. Read the plate at 560 nm (Acceptable Range 540-580 nm.).

CALCULATION OF RESULTS

Average the duplicate OD readings for each calibrator and sample. Create a calibration curve by reducing the data using computer software capable of generating a four-parameter logistic curve (4PLC) fit, after subtracting the mean ODs for the

Zero wells. The sample concentrations obtained should be multiplied by the dilution factor to obtain neat sample values.

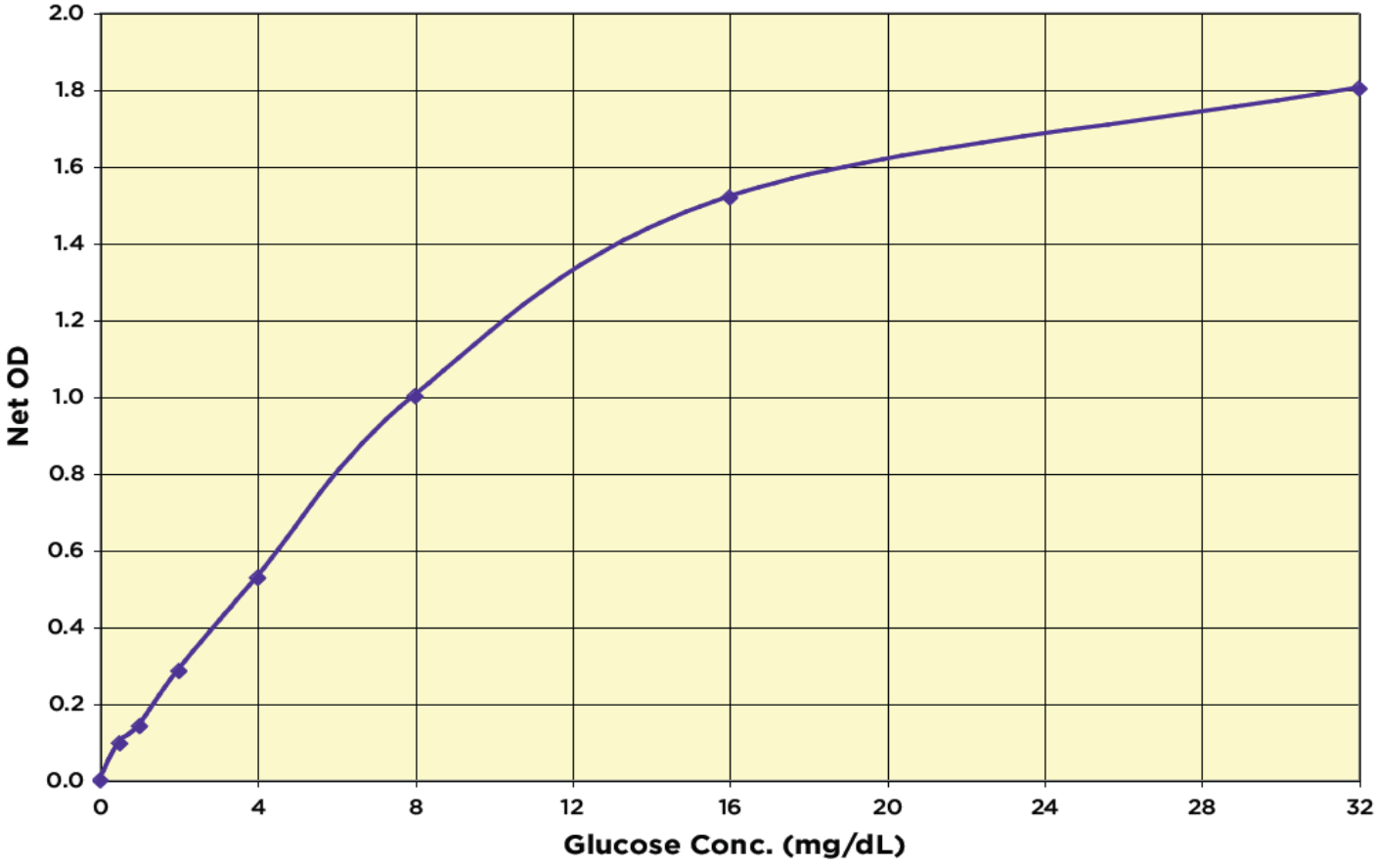
TYPICAL DATA

Sample	Mean OD	Net OD	Glucose Conc. (mg/dL)
Zero	0.058	0.000	0
Standard 1	1.861	1.803	32
Standard 2	1.577	1.519	16
Standard 3	1.060	1.002	8
Standard 4	0.586	0.528	4
Standard 5	0.344	0.286	2
Standard 6	0.200	0.142	1
Standard 7	0.155	0.097	0.5
Sample 1	1.451	1.393	13.4
Sample 2	0.270	0.212	1.6

Always run your own calibration curves for calculation of results. Do not use these data.

Conversion Factor: 100 mg/dL of Glucose is equivalent to 1 mg/mL or 5.51 mM.

Typical Calibration Curve



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VALIDATION DATA

Sensitivity and Limit of Detection

Sensitivity was calculated by comparing the ODs for twenty wells run for each of the zero and calibrator #7. The detection limit was determined at two (2) standard deviations from the zero along the calibration curve.
Sensitivity was determined as 0.413 mg/dL.

The Limit of Detection was determined in a similar manner by comparing the ODs for twenty wells run for each of the zero and a low concentration human sample.
The Limit of Detection was determined as 0.304 mg/dL.

Linearity

Linearity was determined in human serum and plasma samples by taking two diluted samples with known glucose concentrations. One serum sample had a high glucose concentration of 10.7 mg/dL and one had a lower value of 3.67 mg/dL. One plasma sample had a high glucose concentration of 5.42 mg/dL and one had a lower value of 2.25 mg/dL. They were mixed in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used.

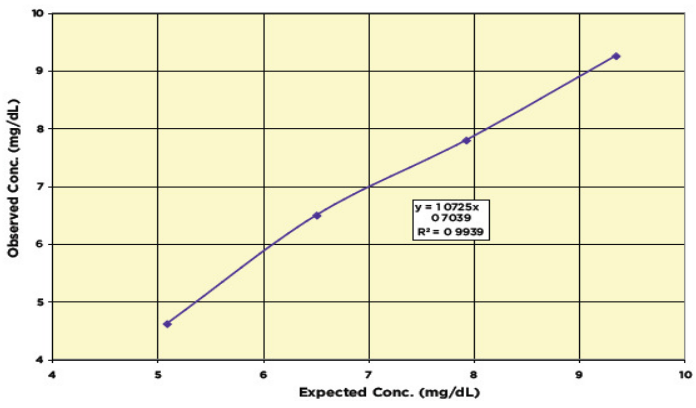
Serum Linearity

Low Sample	High Sample	Observed Conc. (mg/dL)	Expected Conc. (mg/dL)	% Recovery
80%	20%	4.61	5.09	90.6
60%	40%	6.49	6.51	99.8
40%	60%	7.79	7.93	98.3
20%	80%	9.26	9.35	99.0
			Mean Recovery	**96.9%**

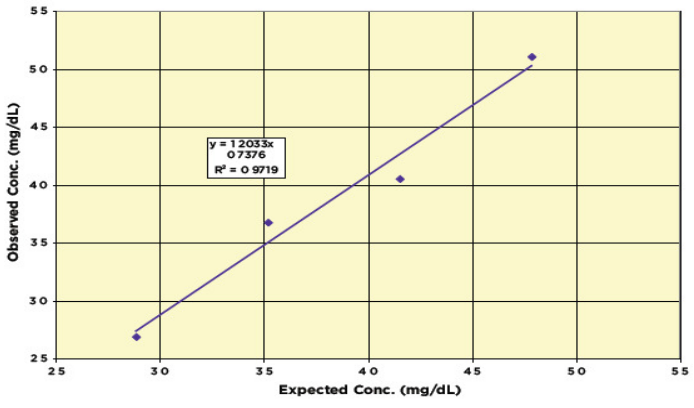
Plasma Linearity

Low Sample	High Sample	Observed Conc. (mg/dL)	Expected Conc. (mg/dL)	% Recovery
80%	20%	2.69	2.89	93.2
60%	40%	3.68	3.52	104.4
40%	60%	4.05	4.15	97.5
20%	80%	5.10	4.79	106.6
			Mean Recovery	**100.4%**

Serum Linearity



Plasma Linearity



Intra Assay Precision
Three diluted human serum samples were run in replicates of 20 in an assay. The mean and precision of the calculated concentrations were:

Sample	Glucose Conc. (mg/dL)	%CV
1	13.96	4.1
2	9.54	3.4
3	1.78	10.5

Inter Assay Precision
Three diluted human serum samples were run in duplicate in seventeen assays run over multiple days by three operators. The mean and precision of the calculated concentrations were:

Sample	Glucose Conc. (mg/dL)	%CV
1	13.19	11.2
2	9.40	6.4
3	1.59	9.4

SAMPLE VALUES

Multiple human serum and plasma samples were tested in the assay at dilutions from 1:10 to 1:60 fold. Adjusted glucose concentrations ranged from 36.7 to 246.7 mg/dL with an average value of 104.0 mg/dL. Tietz states adult serum glucose levels of 70-105 mg/dL, child values of 60-100 mg/dL, with premature babies having levels at 20-60 mg/dL. CSF levels should be 40-70 mg/dL for adults and 60-80 for infants.

FOR RESEARCH USE ONLY

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