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ARBOR
ASSAYS

**MOST
SENSITIVE**

DetectX[®]
Glucose
Fluorescent Detection Kit

2 Plate Kit

Catalog Number K039-F1

SPECIES INDEPENDENT

Sample Types Validated:

Serum, Plasma, Urine, Buffers and TCM

Please read this insert completely prior to using the product.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Not for Human Diagnostic Use

www.ArborAssays.com

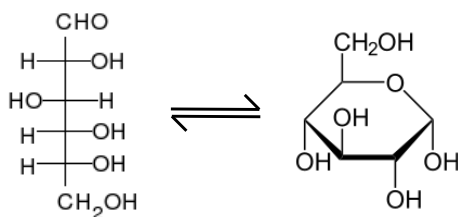
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Glucose ($C_6H_{12}O_6$) is by far the most common carbohydrate. It is a monosaccharide, an aldose, a hexose, and a reducing sugar and is also known as dextrose, because it is dextrorotatory (rotates polarized light clockwise). The structure of glucose 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 repair¹.

A serious fall in blood glucose can be characterized by metabolic dysfunction, neuroglycopenia, seizure, and death². A persistent elevation in blood glucose leads to "glucose toxicity." Glucose toxicity contributes to β -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.

1. Klein, A. and Ferrante, R. "The neuroprotective role of creatine. In Creatine and Creatine Kinase in Health and Disease". Salomons, G.S., Wyss, M., Eds.; Springer: Berlin, , 2007; Vol. 46, 205-243.
2. Wasserman, DH., "Four grams of glucose"., Am. J. Physiol. Endocrinol. Metab. 2009, E11-E21.

The DetectX® Glucose Fluorescent Detection Kit is designed to quantitatively measure glucose in a variety of samples. Please read the complete kit insert before performing this assay. A β -D-glucose standard is provided to generate a standard curve for the assay and all samples should be read off the standard curve. Samples are mixed with the 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, converts the substrate into a fluorescent product. The fluorescent product is read at 590 nm with excitation at 570 nm. Increasing levels of glucose cause a linear increase in fluorescence.

Related Products

DetectX® Kits

Hemoglobin Detection Dual-Range Kit	Catalog Number K013-H1
Urea Nitrogen (BUN) Detection Kit	Catalog Number K024-H1
Urinary Creatinine Detection Kits	Catalog Number K002-H1/H5

Supplied Components

Black 96 well Half Area Plates Corning Costar Plate 3694.	2 Plates	Catalog Number X037-2EA
Glucose Standard Glucose at 100 mg/dL in a special stabilizing solution.	90 μ L	Catalog Number C141-90UL
Assay Buffer Assay buffer containing detergents and stabilizers.	50 mL	Catalog Number X117-50ML
Substrate A solution of the substrate in a special stabilizing buffer.	5 mL	Catalog Number C129-5ML
Horseradish Peroxidase Concentrate A 100X concentrated solution of HRP in a special stabilizing solution.	60 μ L	Catalog Number X107-60UL
Glucose Oxidase Concentrate A 10X concentrated solution of Glucose Oxidase in a special stabilizing solution.	600 μ L	Catalog Number C137-600UL

Storage Instructions

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



OTHER MATERIALS REQUIRED

Repeater pipet with disposable tips capable of dispensing 25 μ L.

96 well plate reader capable of reading fluorescence at 580-590 nm with excitation at 570-580 nm. Set plate parameters for a 96-well Corning Costar 3694 plate. See: <http://www.ArborAssays.com/resources/lit.asp> for plate dimension data.

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°C or lower, preferably after being frozen in liquid nitrogen. Serum and plasma samples can be used after being diluted $\geq 1:15$. Urine samples can be used after being diluted $\geq 1:2$. This assay has been validated for serum, plasma, urine, 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	28 μL	55 μL
Assay Buffer	1.485 mL	2.772 mL	5.445 mL
Total Volume	1.5 mL	2.8 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

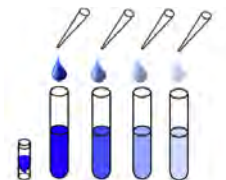
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Standard Preparation

Glucose Standards are prepared by labeling seven tubes as #1 through #7. Briefly vortex to mix the vial of Glucose Standard. 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 Standard 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 10, 5, 2.5, 1.25, 0.625, 0.313, and 0.156 mg/dL.

Use all Standards 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 6
Vol of Addition (μL)	15	75	75	75	75	75	75
Final Conc (mg/dL)	10	5	2.5	1.25	0.625	0.313	0.156

ASSAY PROTOCOL

Use the plate layout sheet on the back page to aid in proper sample and standard identification. Set plate parameters for a 96-well Corning Costar 3694 plate. See: <http://www.ArborAssays.com/resources/lit.asp> for plate dimension data.

1. Pipet 20 μL of diluted samples or standards into duplicate wells in the plate.
2. Pipet 20 μL of Assay Buffer into duplicate wells as the Zero standard.
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 fluorescent emission at 585 ± 5 nm with excitation at 575 ± 5 nm. Please contact your plate reader manufacturer for suitable filter sets.

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CALCULATION OF RESULTS

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Average the duplicate FLU readings for each standard and sample. Create a standard curve by reducing the data using computer software capable of generating a four-parameter logistic curve (4PLC) fit, after subtracting the mean FLU for the Zero wells. The sample concentrations obtained should be multiplied by the dilution factor to obtain neat sample values.

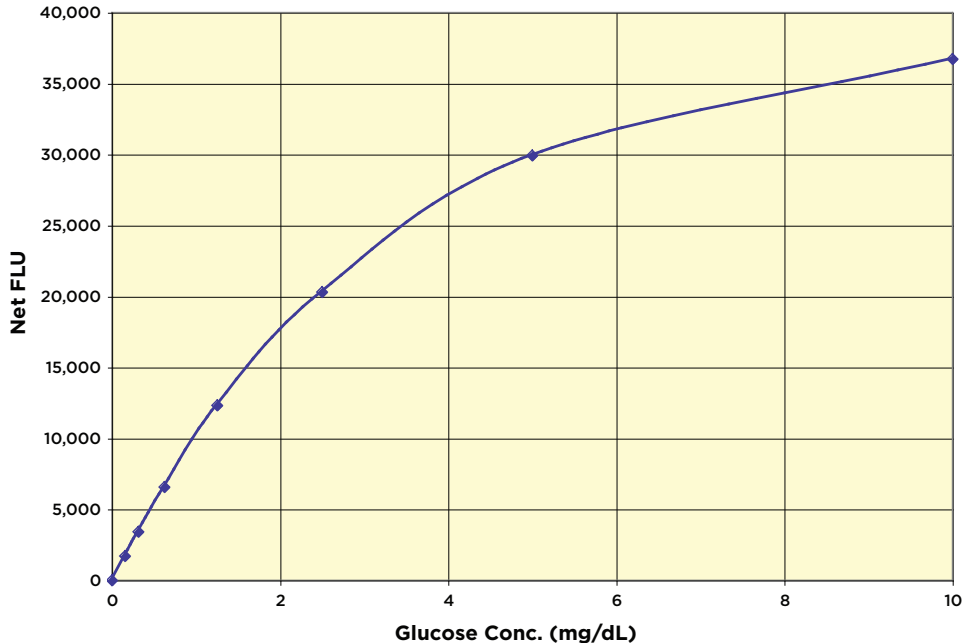
TYPICAL DATA

Sample	Mean FLU	Net FLU	Glucose Conc. (mg/dL)
Zero	867	0	0
Standard 1	37,595	36,728	10
Standard 2	30,806	29,939	5
Standard 3	21,167	20,300	2.5
Standard 4	13,197	12,330	1.25
Standard 5	7,449	6,582	0.625
Standard 6	4,284	3,416	0.313
Standard 7	2,572	1,705	0.156
Sample 1	29,137	28,269	4.47
Sample 2	7,153	6,285	0.602

**Always run your own standard 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 Standard Curve



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

VALIDATION DATA

Sensitivity and Limit of Detection

Sensitivity was calculated by comparing the FLUs for twenty wells run for each of the zero and standard #7. The detection limit was determined at two (2) standard deviations from the zero along the standard curve.

Sensitivity was determined as 8.24 $\mu\text{g}/\text{dL}$. Equivalent to 0.00824 mg/dL.

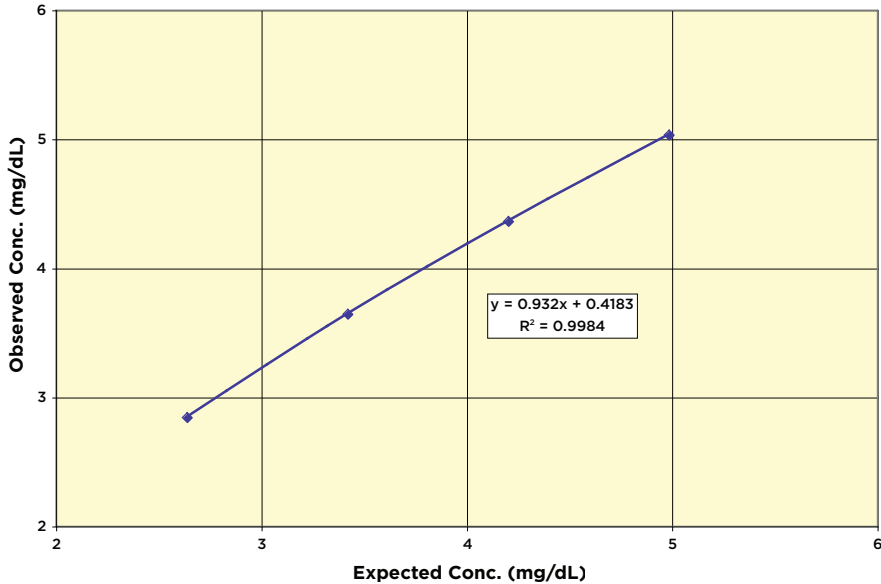
The Limit of Detection was determined in a similar manner by comparing the FLUs for twenty wells run for each of the zero and a low concentration human sample.

The Limit of Detection was determined as 1.42 $\mu\text{g}/\text{dL}$. Equivalent to 0.00142 mg/dL.

Linearity

Linearity was determined in human serum samples by taking two diluted samples with known glucose concentrations. One serum sample had a high glucose concentration of 5.77 mg/dL and one had a lower value of 1.86 mg/dL. They were mixed in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used.

Low Sample	High Sample	Observed Conc. (mg/dL)	Expected Conc. (mg/dL)	% Recovery
80%	20%	2.84	2.64	107.8
60%	40%	3.64	3.42	106.5
40%	60%	4.36	4.20	103.8
20%	80%	5.03	4.99	101.0
			Mean Recovery	104.8%



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	5.03	5.3
2	1.90	2.5
3	0.64	1.9

Inter Assay Precision

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

Sample	Glucose Conc. (mg/dL)	%CV
1	4.51	13.3
2	1.86	6.2
3	0.65	8.3

SAMPLE VALUES

Multiple human serum samples were tested in the assay at dilutions from 1:10 to 1:300 fold. Adjusted glucose concentrations ranged from 37.1 to 119.9 mg/dL with an average value of 69.34 mg/dL. Tietz³ 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.

Multiple human urine samples were tested in the assay at dilutions from 1:2 to 1:20 fold. Adjusted glucose concentrations ranged from 0.35 to 1.77 mg/dL with an average value of 1.152 mg/dL. Tietz³ states normal urine glucose levels of <500 mg/dL

3. Tietz, NW, Textbook of Clinical Chemistry, WB Saunders Company, Philadelphia.

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LIMITED WARRANTY

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Arbor Assays warrants that at the time of shipment this product is free from defects in materials and workmanship. This warranty is in lieu of any other warranty expressed or implied, including but not limited to, any implied warranty of merchantability or fitness for a particular purpose.

We must be notified of any breach of this warranty within 48 hours of receipt of the product. No claim shall be honored if we are not notified within this time period, or if the product has been stored in any way other than outlined in this publication. The sole and exclusive remedy of the customer for any liability based upon this warranty is limited to the replacement of the product, or refund of the invoice price of the goods.

CONTACT INFORMATION

For details concerning this kit or to order any of our products please contact us:

Arbor Assays

1514 Eisenhower Place
Ann Arbor, Michigan 48108 USA
Phone: 734-677-1774
Fax: 734-677-6860
Web: www.ArborAssays.com

E Mail Addresses:

Info@ArborAssays.com
Orders@ArborAssays.com
Technical@ArborAssays.com
Contracts@ArborAssays.com

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