ARBOR ASSAYS™
Interactive Assay Solutions™

MULTI SPECIES

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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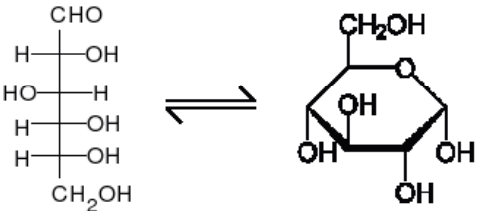
BACKGROUND

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[1].

A serious fall in blood glucose can be characterized by metabolic dysfunction, neuroglycopenia, seizure, and death[2]. 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.

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.

ASSAY PRINCIPLE

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 emission is at 590 nm with excitation at 520 nm. Increasing levels of glucose cause a linear increase in fluorescence.

RELATED PRODUCTS

Kits	Catalog No.
Galactose Colorimetric Detection Kit	K042-H1
Glucose Colorimetric Detection Kit	K039-H1
Hemoglobin High Sensitivity Colorimetric Detection Kit	K013-H1X/H5X
Insulin EIA Kit	K046-H1
Thyroxine (T_4) EIA Kits	K051-H1/H5
Triiodothyronine (T_3) EIA Kit	K056-H1/H5
Urea Nitrogen (BUN) Detection Kit	K024-H1
Urinary Creatinine Detection Kits	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 µL — 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 µL — Catalog Number X107-60UL

Glucose Oxidase Concentrate
A 10X concentrated solution of Glucose Oxidase in a special stabilizing solution.
600 µL — Catalog Number C137-600UL

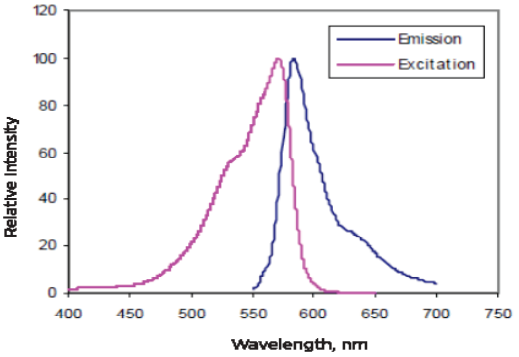
STORAGE INSTRUCTIONS

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 µL.

96 well plate reader capable of reading fluorescence. Optimal signal will be obtained with emission at 590 nm and excitation at 570 nm, however other combinations of excitation and emission wavelengths should be considered. Due to the proximity of the excitation and emission wavelengths for the probes, less restrictive 520 nm emission can also be used. Please see the figures below for the excitation and emission data. The output of signal changes based on filters selected, but the ratio of signal is similar.



Ex/Em Wavelength	High FLU	%High/Low Signal
520/590	27,410	22%
530/620	9,350	23%
544/620	9,870	22%
584/620	7,120	21%
584/665	502	20%

Example of high signal and signal ratio across various wavlength combinations.

Note: Filter bandwidth must be considered when selecting em/ex wavelength

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 ≥ 1:15. Urine samples can be used after being diluted ≥ 1:2. This assay has been validated for serum, plasma, urine, buffer and media samples.

REAGENT PREPARATION

Allow the kit reagents to come to room temperature for 30 minutes. Ensure all samples have reached room temperature and have been diluted as approproate prior to running them in the kit.

Horseradish Peroxidase (HRP) and Glucose Oxidase (GOD) Preparation

Dilute the HRP Stock solution 1:100 with Assay Buffer using the table below:

	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

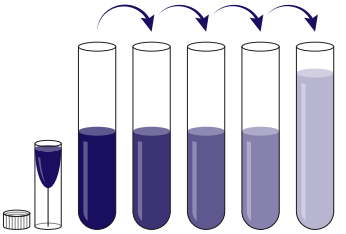
Dilute the GOD Stock solution 1:10 with Assay Buffer using the table below:

	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

Both reagents are stable for at least 3 hours after dilution.

Standard Preparation

Glucose Standards are prepared by labeling 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

We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine Glucose concentrations.

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: www.arborassays.com/resources/#general-info 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 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 with proper wavelength excitation. See page 5 for emission and excitation spectra. We recommend emission at 590 nm with excitation at 520 nm. Please contact your plate reader manufacturer for suitable filter sets.

CALCULATION OF RESULTS

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.

Or use the online tool from MyAssays to calculate the data:
www.myassays.com/arbor-assays-glucose-fluorescent-detection-kit.assay

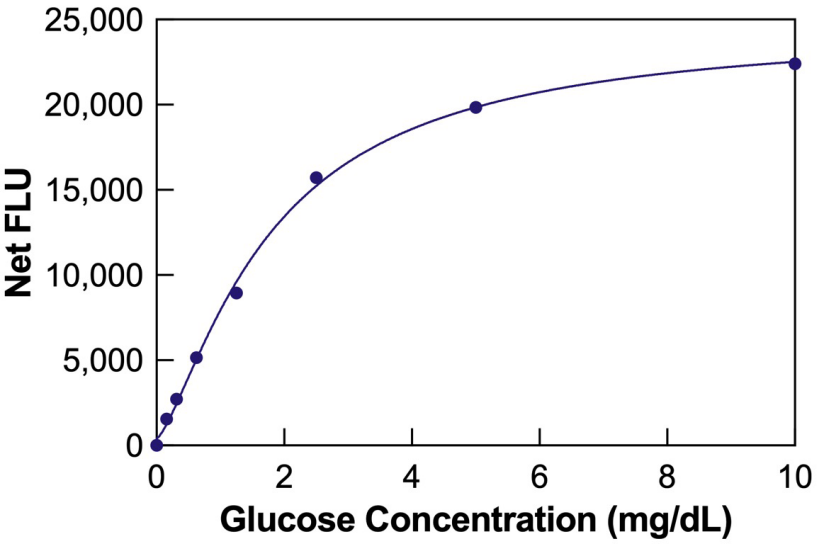
TYPICAL DATA

Sample	Mean FLU	Net FLU	Glucose Conc. (mg/dL)
Zero	2,646	0	0
Standard 1	25,055	22,409	10
Standard 2	22,387	19,841	5
Standard 3	18,374	15,728	2.5
Standard 4	11,585	8,939	1.25
Standard 5	7,793	5,146	0.625
Standard 6	5,372	2,725	0.313
Standard 7	4,196	1,549	0.156
Sample 1	22,424	19,777	4.477
Sample 2	7,726	5,080	0.65

Always run your own standard curves for calculation of results. Do not use this 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 µg/dL. Equivalent to 0.00824 mg/dL.

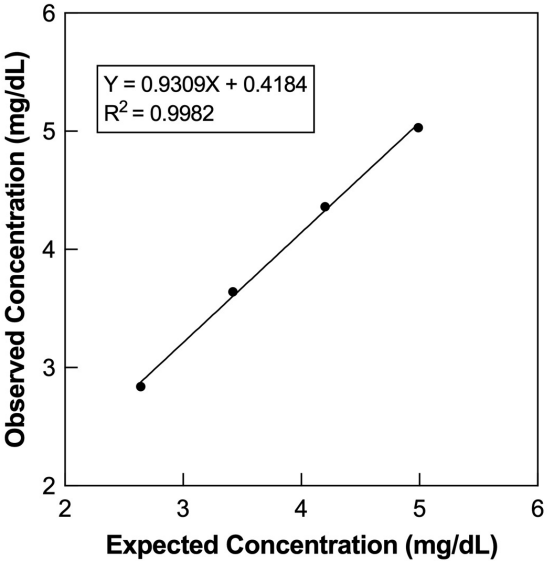
The Limit of Detection was determined in a similar manner by comparing the FLUs for twenty wells run for each of the zero and a low concentration human sample.

The Limit of Detection was determined as 1.42 µg/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	Expected Conc. (mg/dL)	Observed Conc. (mg/dL)	% Recovery
80%	20%	2.64	2.84	107.8
60%	40%	3.42	3.64	106.5
40%	60%	4.20	4.36	103.8
20%	80%	4.99	5.03	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. Adjusted glucose concentrations ranged from 37.1 to 119.9 mg/dL with an average value of 69.34 mg/dL. Tietz[3] 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. Tietz[3] states normal urine glucose levels of < 500 mg/dL

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

LIMITED WARRANTY

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

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OFFICIAL SUPPLIER TO ISWE

Arbor Assays and the International Society of Wildlife Endocrinology (ISWE) signed an exclusive agreement for Arbor Assays to supply ISWE members with ELISA kits for wildlife conservation research.