Glucose (Glc)

Colorimetric/Fluorometric Assay kit

(96 Tests)

Zellbio GmbH (Germany)

CAT No. ZX-77100-96

www.zellx.de

Sample Types Validated for:

Serum, Plasma, Urine, Buffers and TCM

!!! Caution: This product is for Research Use Only. Not for *in vitro* Diagnostics !!!

Table of Contents

Introduction 3

Background 3

Assay principle 3

General information 4

Materials supplied in the Kit 4

Storage instruction 4

Materials required but not supplied 4

Precautions 4

General remarks 4

Assay protocol 5

Reagent preparation 5

Sample preparation 5

Standard preparation 6

Assay Procedure 8

Calculation 8

Assay range 9

Sensitivity 10

Precision 10

Protocol summary Colorimetric 11

Protocol summary Fluorometric 12

References 13

Please read this insert completely prior to using the product.

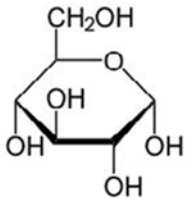
Introduction

Background

Glucose ($C_6H_{12}O_6$) is the most abundant carbohydrate, which belongs to the group of monosaccharide (simple sugars). It is an aldose, a hexose, and a reducing sugar. The D-Glucose, also known as dextrose, is a dextrorotatory compound (i.e. rotating polarized light clockwise) that widely exists in nature, as opposed to the L-Glucose isomer which rarely occurs.

All living organisms can use Glucose as a major source of energy to produce adenosine triphosphate (ATP) for their biological and molecular activities and multiple cellular functions. In other words, most energy in biological system is generated through aerobic cellular respiration of carbohydrate and Glucose. Reduced energy levels threaten cellular homeostasis and integrity, as impaired energy metabolism may trigger apoptosis (programmed cell death), oxidative damage, excitotoxicity and impeded 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 pancreatic 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.



Assay principle

The ZellX® Glucose Colorimetric/Fluorometric assay Kit is designed to quantitatively measure Glucose in a variety of samples including urine, serum, plasma, buffers and TCM. 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 (HRP) and the reaction is 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 Substrate to convert the substrate either into a pink-colored product to be read at 560 nm or a fluorescent product to be read at 590 nm with excitation at 520 nm. Increasing levels of Glucose lead to an increase in color/ fluorescent signal.

General information

Materials supplied in the Kit

Component	*Quantity*
Glucose Standard (320 mg/dL)	45 µL
Glucose Oxidase Concentrate (10 X)	300 µL
Assay Buffer	25 mL
Substrate	2.5 mL
HRP Concentrate (100 X) (Colorimetric)	30 µL
HRP Concentrate (100 X) (Fluorometric)	30 µL
Clear Half Area 96 Well Plate	1 plate
Black Half Area 96 Well Plate	1 plate

Storage instruction

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

Materials required but not supplied

Deionized water (diH_2O)

Microplate/ELISA Reader capable of reading optical absorption at 560 nm (540-580 nm is acceptable)

Microplate Reader capable of reading fluorescent at 590 nm with excitation at 520 nm

Precision pipettes, multichannel pipette and disposable pipette tips

Disposable 1.5-2 mL microtubes for sample preparation

Precautions

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.

General remarks

- Equilibrate all kit components to room temperature (RT) 30 minutes before use.

- The instruction must be strictly followed. The reading of Microplate/ELISA Reader must be set at the appropriate wavelength for determining the experiment result.
- Pipette tips should not be used more than once in order to avoid cross contamination.
- Reagents of different batches should not be mixed or used after their expiration dates.

Assay protocol

Reagent preparation

i. **Glucose Oxidase Working Solution:** Prepare a 1:10 dilution of Glucose Oxidase Concentrate with Assay Buffer (1 part Glucose Oxidase Conc. with 9 parts Assay Buffer), and mix well.

ii. **HRP Reagent:** Vortex the suspension of HRP Concentrate **(Colorimetric or Fluorometric),** prior to pipetting, and then add 25 µL of HRP Concentrate to 2.475 mL of Assay Buffer and mix well (1:100 dilution).

- **Important Note: There are 2 different HRP Conc. bottles, which must be prepared separately for colorimetric and fluorometric assays.**

Sample preparation

After collecting the sample, extraction should be immediately carried out in accordance with related instruction. After extraction, experiment should be conducted immediately, otherwise, keep the sample at -70 or -80˚C; preferably after being frozen in liquid nitrogen. **Avoid repeated freeze-thaw cycles.**

I. **Serum & Plasma:**

- Serum and plasma should be diluted ≥ 1:15 by taking one part of serum and adding 14 or more parts of Assay Buffer prior to conducting the assay.

II. **Urine:**

- Urine sample should be diluted ≥ 1:2 by taking one part of urine and adding 1 or more parts of Assay Buffer prior to conducting the assay.

III. **Buffer & TCM:**

- For measuring Glucose in Buffer and tissue culture media (TCM), samples should be read off a standard curve generated in corresponding Buffer or TCM.

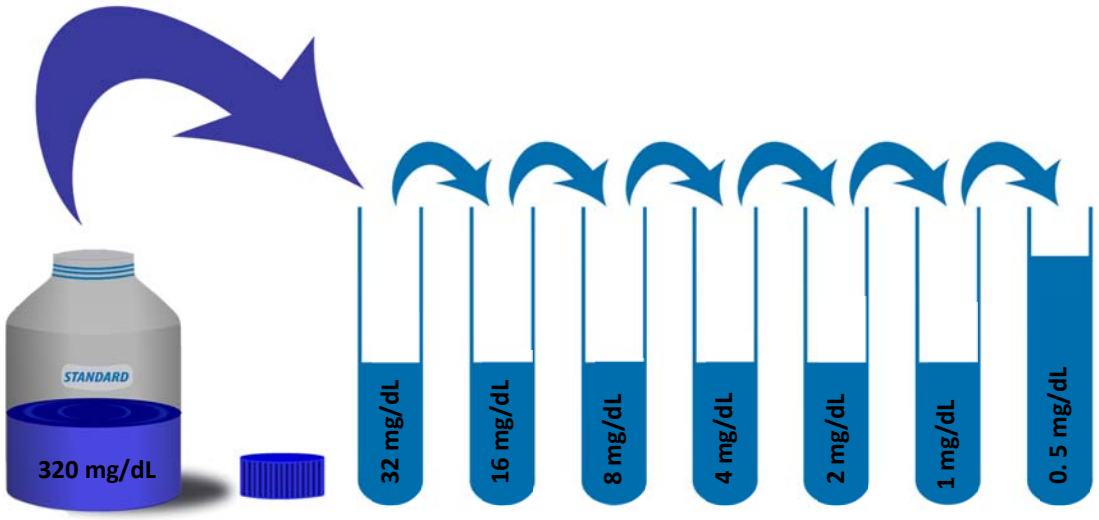
All samples must be used immediately after dilution.

Standard preparation

For colorimetric measurement

- Prepare a 1:10 dilution of Glucose Standard with Assay Buffer (mix 20 µL of standard with 180 µL of Assay Buffer), and label as the Standard No.7 (32 mg/dL).
- Apply series of other dilutions as described in the table.
- The Assay Buffer is used as the 0 mg/dL standard.

No.	*Concentration*	*Material needed*
Standard No.7	32 mg/dL	20 µL Glucose Standard + 180 µL Assay Buffer
Standard No.6	16 mg/dL	100 µL Standard No.7 + 100 µL Assay Buffer
Standard No.5	8 mg/dL	100 µL Standard No.6 + 100 µL Assay Buffer
Standard No.4	4 mg/dL	100 µL Standard No.5 + 100 µL Assay Buffer
Standard No.3	2 mg/dL	100 µL Standard No.4 + 100 µL Assay Buffer
Standard No.2	1 mg/dL	100 µL Standard No.3 + 100 µL Assay Buffer
Standard No.1	0.5 mg/dL	100 µL Standard No.2 + 100 µL Assay Buffer
Standard No.0	0 mg/dL	100 µL Assay Buffer

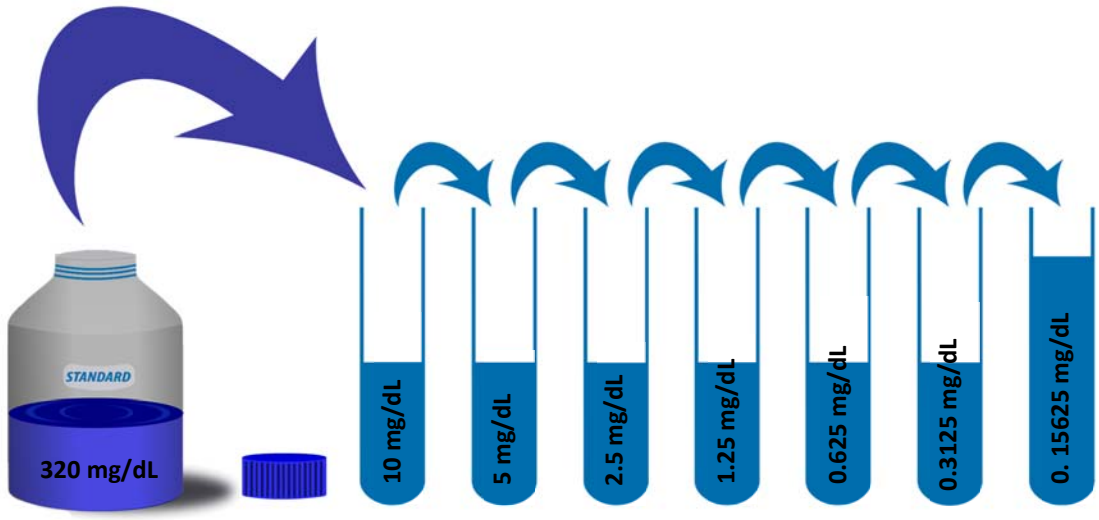


All standard must be used within 2 hours of preparation

For Fluorometric measurement

- Prepare a 1:32 dilution of Glucose Standard with Assay Buffer (mix 10 µL of standard with 310 µL of Assay Buffer), and label as the Standard No.7 (10 mg/dL).
- Apply series of other dilutions as described in the table.
- The Assay Buffer is used as the 0 mg/dL standard.

No.	*Concentration*	*Material needed*
Standard No.7	10 mg/dL	10 µL Glucose Standard + 310 µL Assay Buffer
Standard No.6	5 mg/dL	100 µL Standard No.7 + 100 µL Assay Buffer
Standard No.5	2.5 mg/dL	100 µL Standard No.6 + 100 µL Assay Buffer
Standard No.4	1.25 mg/dL	100 µL Standard No.5 + 100 µL Assay Buffer
Standard No.3	0.625 mg/dL	100 µL Standard No.4 + 100 µL Assay Buffer
Standard No.2	0.3125 mg/dL	100 µL Standard No.3 + 100 µL Assay Buffer
Standard No.1	0.15625 mg/dL	100 µL Standard No.2 + 100 µL Assay Buffer
Standard No.0	0 mg/dL	100 µL Assay Buffer



All standard must be used within 2 hours of preparation

Assay Procedure

For colorimetric measurement

- Pipette 20 µL of either samples or standards into duplicate wells in the **clear half area 96 well-plate**.
- Pipette 20 µL of Assay Buffer as the Zero standard.
- **Add 25 µL of the Colorimetric HRP Reagent to each well using a multichannel pipette.**
- Add 25 µL of the Substrate Solution to each well using a multichannel pipette.
- Add 25 µL of the Glucose Oxidase Working Solution to each well using a multichannel pipette.
- Incubate at room temperature for 30 minutes.
- Read the optical density at 560 nm. (540-580 nm)

For Fluorometric measurement

- Pipette 20 µL of either samples or standards into duplicate wells in the **Black half area 96 well-plate**.
- Pipette 20 µL of Assay Buffer as the Zero standard.
- **Add 25 µL of the Fluorometric HRP Reagent to each well using a multichannel pipette.**
- Add 25 µL of the Substrate Solution to each well using a multichannel pipette.
- Add 25 µL of the Glucose Oxidase Working Solution to each well using a multichannel pipette.
- Incubate at room temperature for 30 minutes.

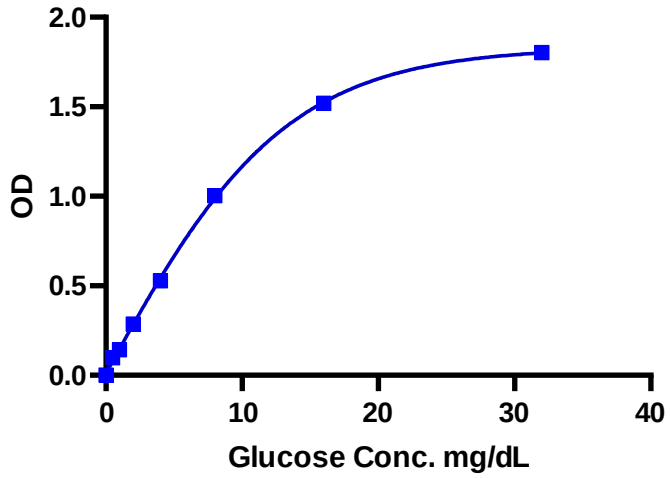
  Read the fluorescent intensity at 590 nm with the excitation at 520 nm.

Calculation

- Average the duplicate optical density (OD)/ Fluorescent Unit (FLU) readings for each standard and sample.
- Create a standard curve by reducing the data using the four parameter logistic curve (4PLC) fitting routine on the plate reader using the adjusted OD/FLU values
- The concentrations obtained should be multiplied by the dilution factor to obtain sample values.

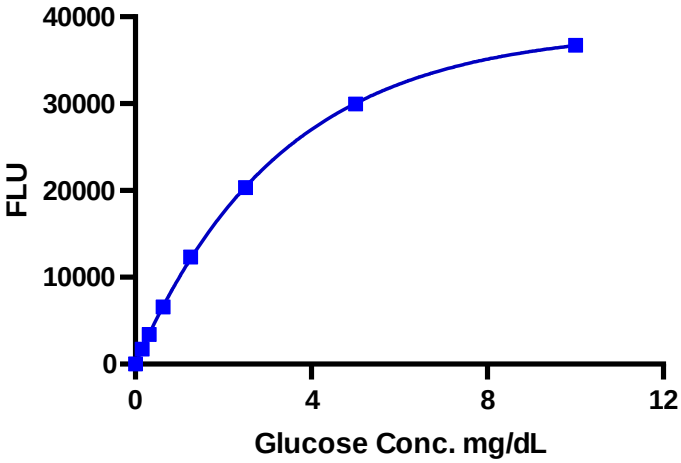
Conversion Factor:

100 mg/dL of Glucose is equivalent to 5.51 mM.



A typical standard curve of ZellX® Glucose Colorimetric Assay

Run your own standard curves for calculation of results



A typical standard curve of ZellX® Glucose Fluorometric Assay

Run your own standard curves for calculation of results

Assay range

The limit of detection of ZellX® Glucose assay was determined as **0.304 mg/dL** for Colorimetric method and **1.42 µg/dL** for Fluorometric method.

Sensitivity

The sensitivity of the ZellX® Glucose assay was determined as **0.413 mg/dL** for Colorimetric method and **8.24 µg/dL** for Fluorometric method.

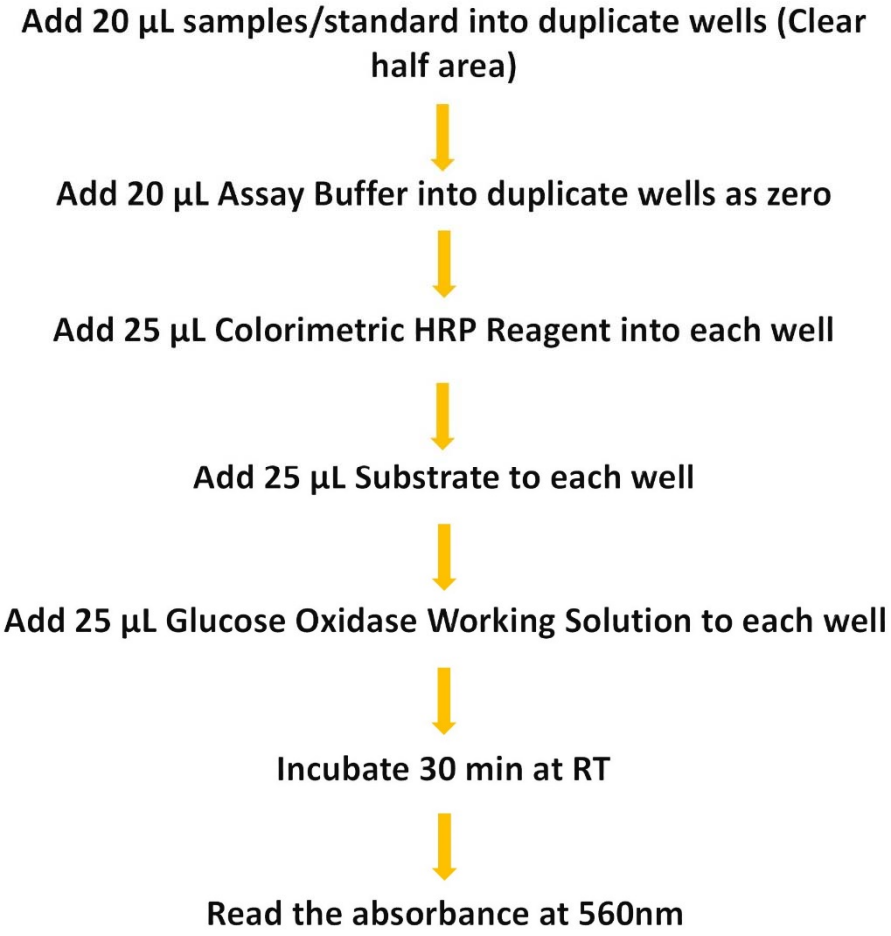
Precision

Intra-Assay Precision (Precision within an assay): 3 human serum samples were tested 20 times (both Colorimetric & Fluorometric) in an assay.

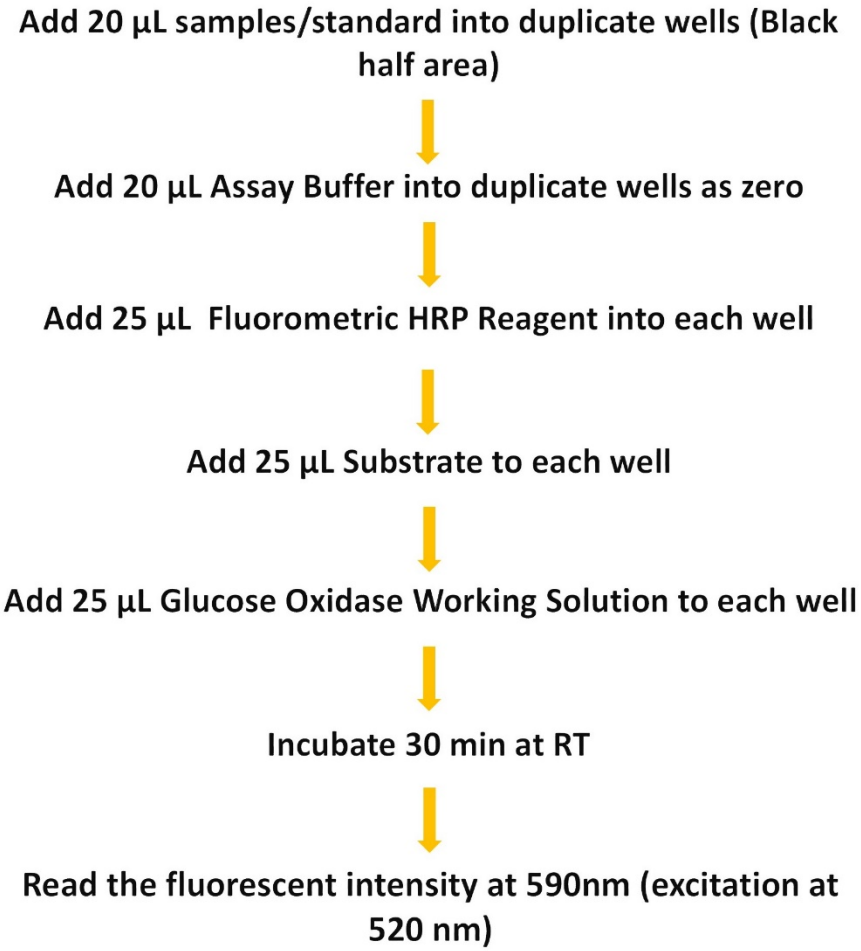
Inter-Assay Precision (Precision between assays): 3 human serum samples were tested in duplicate on 17 different assays (Colorimetric) & 12 different assays (Fluorometric) over multiple days.

Item	*%CV Colorimetric*	*%CV Fluorometric*
Intra assay	3.4, 4.1, 10.5	2.5, 1.9, 5.3
Inter assay	6.4, 9.4, 11.2	6.2, 8.3, 13.3

Protocol summary Colorimetric



Protocol summary Fluorometric



References

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.