Glucose Colorimetric Assay Kit

Cat#: orb1173245 (ELISA Manual)

Micro Glucose Assay Kit
Cat #: orb1173245 Size: 96 T/192 T
Lot #: Refer to product label
Detection range: 4-300 mg/dL **Sensitivity:** 4 mg/dL
Applicable samples: Animal and Plant Tissues, Cells, Bacteria, Serum, Plasma, Urine, or other Biological Fluids
Storage: Stored at -20℃ for 12 months

Assay Principle

Glucose (C6H12O6, FW: 180.16) is the primary biological fuel source used to generate the universal energy molecule, ATP. Due to its importance in metabolism, glucose level is a key diagnostic parameter for many metabolic disorders. Abnormal glucose levels have been associated with several metabolic dysfunctions such as hypoglycemia, hyperglycemia, and diabetes mellitus. Measurements of glucose levels in tissues and body fluids (such as blood and urine) are often used for the diagnosis of glucose related disorders. Glucose levels are also monitored to check the efficacy of therapeutics such as insulin and sulfonylureas in type 2 diabetics. Micro Glucose Assay Kit provides a simple method for detecting Glucose concentration in various biological samples, including serum, plasma, urine, other body fluid, food, growth medium, etc. The principle is that the improved o-Toluidine method utilizes a specific color reaction with glucose. The absorbance at 630 nm is directly proportional to glucose concentration in the sample.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	96 T	192 T	
o-Toluidine Reagent	60 mL	120 mL	4℃
Glucose Standard (300 mg/dL)	1 mL	2 mL	-20℃

Materials Required but Not Supplied

·Microplate reader or visible spectrophotometer capable of measuring absorbance at 630 nm
·96-well plate or microglass cuvette
·Centrifuge, water bath
·Precision pipettes, disposable pipette tips
·Deionized water, PBS
·Dounce homogenizer (for tissues)

Reagent Preparation

o-Toluidine Reagent: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.
Glucose Standard (300 mg/dL): Ready to use as supplied. Equilibrate to room temperature before use. Store at -20℃.
Setting of standard curves: Dilute the 300 mg/dL Glucose Standard to 300, 200, 100, 50, 20, 10, 4 mg/dL standard with deionized water, as shown in the following table.

Num.	Volume of 300 mg/dL Standard (μL)	Volume of Deionized Water (μL)	Standard Concentration (mg/dL)
Std.1	150	0	300
Std.2	100	50	200
Std.3	50	100	100
Std.4	25	125	50
Std.5	10	140	20
Std.6	5	145	10
Std.7	2	148	4

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for 1 month.

1. Animal Tissues: Weigh 0.1 g tissue, add 1 mL PBS and homogenize on ice. Centrifuge at 12,000 g for 5 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Plant Tissues: Weigh 0.1 g tissue, add 1 mL PBS and mash. Ultrasonic break in ice bath 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 12,000 g for 5 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
3. Cells or Bacteria: Collect 5×106 cells or bacteria into the centrifuge tube, wash cells or bacteria with cold PBS, discard the supernatant after centrifugation; add 1 mL PBS to ultrasonically disrupt the cells or bacteria 5 min (power 20% or 200 W, ultrasonic
3 s, interval 7 s, repeat 30 times). Centrifuge at 12,000 g for 5 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
4. Plasma, Serum and Urine (and other biological fluids): Tested directly.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 630 nm. Visible spectrophotometer was returned to zero with deionized water.
2. Sample measurement. (The following operations are operated in the EP tube)

Reagent	Blank Tube (μL)	Standard Tube (μL)	Test Tube (μL)
Supernatant	0	0	25
Standard	0	25	0
Deionized Water	25	0	0
o-Toluidine Reagent	500	500	500

3. Mix well, heat in a boiling water bath for 8 min, and cool down in cold water bath for 4 min. Take 200 μL to 96 well plate or microglass cuvette. The absorbance value (OD value) is measured at 630 nm. The Blank Well is marked as ABlank, the Standard Well is marked as AStandard, and the Test Well is marked as ATest. Finally calculate ΔATest=ATest-ABlank，ΔAStandard=AStandard-ABlank.

Note: Blank Well only needs to measure 1-2 times. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If the ATest values are higher than the OD value for the 300 mg/dL Standard, dilute sample in water and repeat the assay. Multiply the results by the dilution factor (n).

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Drawing of standard curve With the concentration of the standard solution as the y-axis and the ΔAStandard as the x-axis, draw the standard curve.
2. Calculation of the concentration of Glucose Substitute the ΔATest of the sample into the equation to obtain the y value (mg/dL).
(1) Calculated by fresh weight of samples
Glucose content (mg/g fresh weight)=y÷100×VSample÷(W×VSample÷VSample total)×n**=y÷100÷W×n**
(2) Calculated by volume of liquid samples
Glucose content (mg/dL)=y×VSample÷VSample×n**=y×n**
(3) Calculated by number of cells or bacteria
Glucose content (mg/104)=y÷100×VSample÷(500×VSample÷VSample total)×n **=y÷50,000×n**
Where: VSample: add sample volume, 0.025 mL; 100: 1 dL=100 mL; W: weight of sample, g; VSample total: add PBS volume to sample, 1 mL; n: the sample dilution factor; 500: Total number of cells or bacteria, 5×106.

Conversions: 1mg/dL glucose equals 55.5 μM, 0.001% or 10 ppm.
Typical serum/plasma glucose values: 70-110 mg/dL.

Typical Data

Typical standard curve

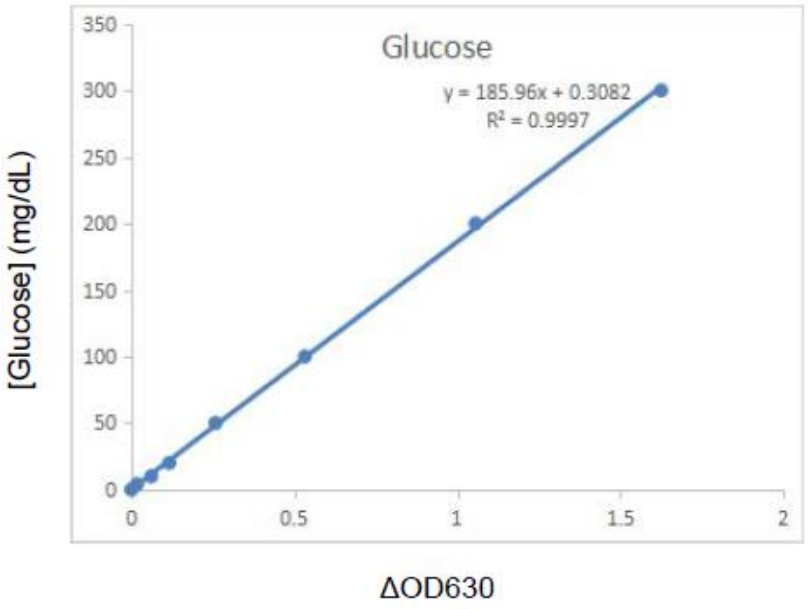

Figure 1. Standard curve of Glucose assay, data provided for demonstration purposes only. A new standard curve must be generated for each assay.