CheKine™ Micro Glucose Assay Kit

Cat #: KTB1300 Size: 96 T/96 S 192 T/192 S

	Micro Glucose Assay Kit		
REF	**Cat #:** KTB1300	LOT	**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 ($C_6H_{12}O_6$, 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. CheKine™ 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	60 mL×2	4℃
Glucose Standard (300 mg/dL)	1 mL	2 mL	-20℃

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

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℃.

Note: o-Toluidine Reagent is toxic. Please wear protective measures such as masks and gloves during the experiment.

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

Note: Always prepare a fresh set of standards per use. Diluted standard solution is unstable and must be used within 4 h.

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 5×10^6 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 (4℃) for 4 min. Take 200 μL to 96 well plate or microglass cuvette. The absorbance value (A value) is measured at 630 nm. The Blank Well is marked as A_{Blank}, the Standard Well is marked as $A_{Standard}$, and the Test Well is marked as A_{Test}. Finally calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

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 A_{Test} values are higher than the ΔA value for the 300 mg/dL Standard, dilute sample in deionized water and repeat the assay. Multiply the results by the dilution factor (n). If the ΔA measurement of the sample is lower than 4 mg/dL, the sample size can be increased.

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 $\Delta A_{Standard}$ as the x-axis, draw the standard curve.

2. Calculation of the concentration of Glucose

Substitute the ΔA_{Test} 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)=$y\div100\times V_{Sample}\div(W\times V_{Sample}\div V_{Sample\ total})\times n$**=$y\div100\div W\times n$**

(2) Calculated by volume of liquid samples

Glucose content (mg/dL)=$y\times V_{Sample}\div V_{Sample}\times n$**=$y\times n$**

(3) Calculated by number of cells or bacteria

Glucose content (mg/10^4)=$y\div100\times V_{Sample}\div(500\times V_{Sample}\div V_{Sample\ total})\times n$ **=$y\div50{,}000\times n$**

Where: V_{Sample}: add sample volume, 0.025 mL; 100: 1 dL=100 mL; W: weight of sample, g; $V_{Sample\ total}$: add PBS volume to sample, 1 mL; n: the sample dilution factor; 500: Total number of cells or bacteria, 5×10^6.

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

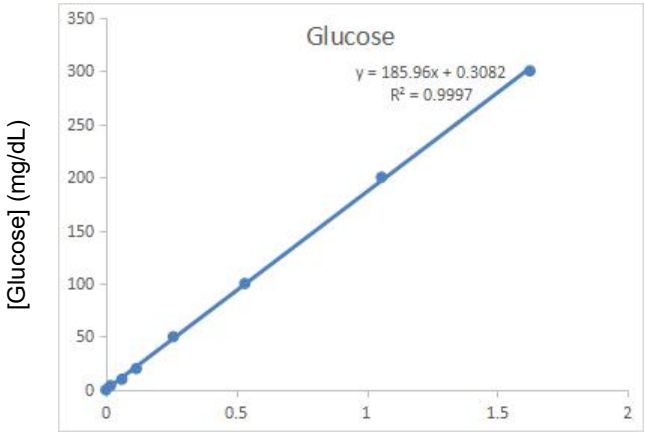

Figure 1. Standard curve of Glucose assay, data provided for demonstration purposes only. A new standard curve must be generated for each assay.

Recommended Products

Catalog No.	Product Name
KTB1350	CheKine™ Micro Total Carbohydrate Assay Kit
KTB1340	CheKine™ Micro Glycogen Assay Kit
KTB1360	CheKine™ Micro Reducing Sugar (RS) Assay Kit
KTB1320	CheKine™ Micro Plant Soluble Sugar Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.