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CheKine™ Pro Glucose Fluorometric Assay Kit

Cat #: KTB9300 Size: 48 T/96 T

	Glucose Fluorometric Assay Kit		
REF	**Cat #:** KTB9300	LOT	**Lot #:** Refer to product label
	Detection range: 0.1-50 μM		**Sensitivity:** 0.1 μM
	Applicable samples: Animal and Plant Tissues, Cells, Serum, Plasma, Saliva, Urine, Body fluid		
	Fluorescence Excitation/Emission: Ex/Em=535/590 nm		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Glucose is the main energy source for virtually all living organisms. Glucose level is a key diagnostic parameter for many metabolic disorders. Measurement of glucose can be very important in both research and drug discovery processes. CheKine™ Pro Glucose Fluorometric Activity Assay Kit can be used to detect biological samples such as animal tissue, cells, serum, plasma, saliva, urine or body fluid. In the kit, glucose oxidase catalyzes the oxidation of glucose into gluconic acid, and produces hydrogen peroxide, under the action of peroxidase, catalyzes the reaction of hydrogen peroxide with non-fluorescent substances to produce fluorescent substances (Ex/Em=535/590 nm), which is proportional to the amount of glucose.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Assay Buffer	100 mL	100 mL×2	-20℃
Reagent Ⅰ	25 μL	50 μL	-20℃, protected from light
Reagent Ⅱ	10 μL	20 μL	-20℃, protected from light
Reagent Ⅲ	50 μL	100 μL	-20℃, protected from light
Standard (400 nmol/mL)	400 μL	400 μL	-20℃, protected from light

Materials Required but Not Supplied

- Fluorescence microplate reader (Ex/Em=535/590 nm)
- 96-well black plate, precision pipettes, disposable pipette tips
- Incubator, ice maker, freezing centrifuge
- Deionized water

• Homogenizer or mortar (for tissue samples)

Reagent Preparation

Assay Buffer: Ready to use as supplied; Equilibrate to room temperature before use; Store at -20℃.

Reagent I: Ready to use as supplied; Equilibrate to room temperature before use; Store at -20℃, protected from light. The remaining reagent can also be stored at -20℃ and protected from light after aliquoting to avoid repeated freezing and thawing.

Reagent II: Ready to use as supplied; Store at -20℃, protected from light.

Reagent III: Ready to use as supplied; Store at -20℃, protected from light.

Working Reagent: For each well, prepare 50 μL Working Reagent which is freshly prepared. Mix evenly according to the ratio of Assay Buffer: Reagent Ⅰ: Reagent Ⅱ: Reagent Ⅲ=4,840 μL: 50 μL: 10 μL: 100 μL.

Standard (400 nmol/mL): Ready to use as supplied; Equilibrate to room temperature before use; Store at -20℃, protected from light. The remaining reagent can also be stored at -20℃ and protected from light after aliquoting to avoid repeated freezing and thawing.

Standard preparation:

4 nmol/mL Standard: Prepare 4 nmol/mL Standard by diluting 10 μL Standard into 990 μL Assay Buffer. Using 4 nmol/mL Standard, prepare standard curve dilution as described in the table:

Num.	Standard Volume (μL)	Assay Buffer (μL)	Concentration (nmol/mL)
Std.1	12.5 μL 4 nmol/mL Standard	987.5	50
Std.2	500 μL of Std.1 (50 nmol/mL)	500	25
Std.3	500 μL of Std.2 (25 nmol/mL)	500	12.5
Std.4	500 μL of Std.3 (12.5 nmol/mL)	500	6.25
Std.5	500 μL of Std.4 (6.25 nmol/mL)	500	3.125
Std.6	500 μL of Std.5 (3.125 nmol/mL)	500	1.5625
Std.7	500 μL of Std.6 (1.5625 nmol/mL)	500	0.78125
Blank	0	500	0

Notes: Always prepare fresh Standards per use; Diluted Std. 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 one month.When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

1. Tissues: Weigh 0.1 g tissue, add 1 mL Assay Buffer and homogenize or mortar on ice. Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

2. Cells: Collect 5×10^6 cells into the centrifuge tube, wash cells or bacteria with cold PBS, discard the supernatant after centrifugation; add 1 mL Assay Buffer 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 10,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

3. Plasma, Serum or other Liquid samples: Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

Before testing, please according to the following table for dilution, the diluent is Assay Buffer(for reference only) :

Carrot	100-300	Rabbit liver	100-400
Mouse muscle	5-20	HT29 cell	5-20

FBS	100-300	Jurkat cell culture medium	50-200
Saliva	1-3	Urine	2-5

Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the fluorescence microplate reader for more than 30 min, and adjust the excitation wavelength to 535 nm and the emission wavelength to 590 nm.
2. Sample measurement. (The following operations are operated in the 96-well black plate)

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)
Sample	0	0	50
Standard	0	50	0
Assay Buffer	50	0	0
Working Reagent	50	50	50

3. Mix well, incubate for 15 min at 37℃. Detect the fluorescence value RFU at Ex/Em=535/590 nm. The Blank Well is recorded as RFU_{Blank}, the standard Well is marked as $RFU_{Standard}$, the Test Well is marked as RFU_{Test}. Finally calculate $\Delta RFU_{Test}=RFU_{Test}-RFU_{Blank}$, $\Delta RFU_{Standard}=RFU_{Standard}-RFU_{Blank}$.

Note: The Blank Well and the Standard Well only need to be done 1-2 times. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples to be diluted into different concentrations. According to the results of the pre-experiment, combined with the linear range of this kit: 0.1-50 μM, the appropriate dilution ratio was selected for sample detection.

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 x-axis and the $\Delta RFU_{Standard}$ as the y-axis, draw the standard curve and obtain the standard equation y=kx+b. The determination of ΔRFU_{Test} is brought into the equation to get x (nmol/mL).

2. Calculation of the Glucose content

(1) Calculated by protein concentration

Glucose (nmol/mg prot)**=x÷Cpr×F**

(2) Calculated by fresh weight of samples

Glucose (nmol/g fresh weight)=$x\div(W\div V_{Total\ sample})\times F$**=x÷W×F**

(3) Calculated by bacteria or cells

Glucose (nmol/10^4 cell)=$x\div(n\div V_{Total\ sample})\times F$**=x÷n×F**

(4) Calculated by volume of liquid samples

Glucose (nmol/mL)**=x×F**

$V_{Total\ sample}$: Added the Assay Buffer volume, 1 mL; Cpr: sample protein concentration, mg/mL; W: Sample weight, g; n: Number of cells, calculated in units of ten thousand.

Precautions

1. Reagent I is photosensitive, use and storage should be strictly protected from light.
2. If the color of Working Reagent appears red, the Standard can be tested first. If the Blank well fluorescence value is greater

than Std.7 (0.78125 nmol/mL), the Working Reagent will be invalid; otherwise, it can continue to be used.

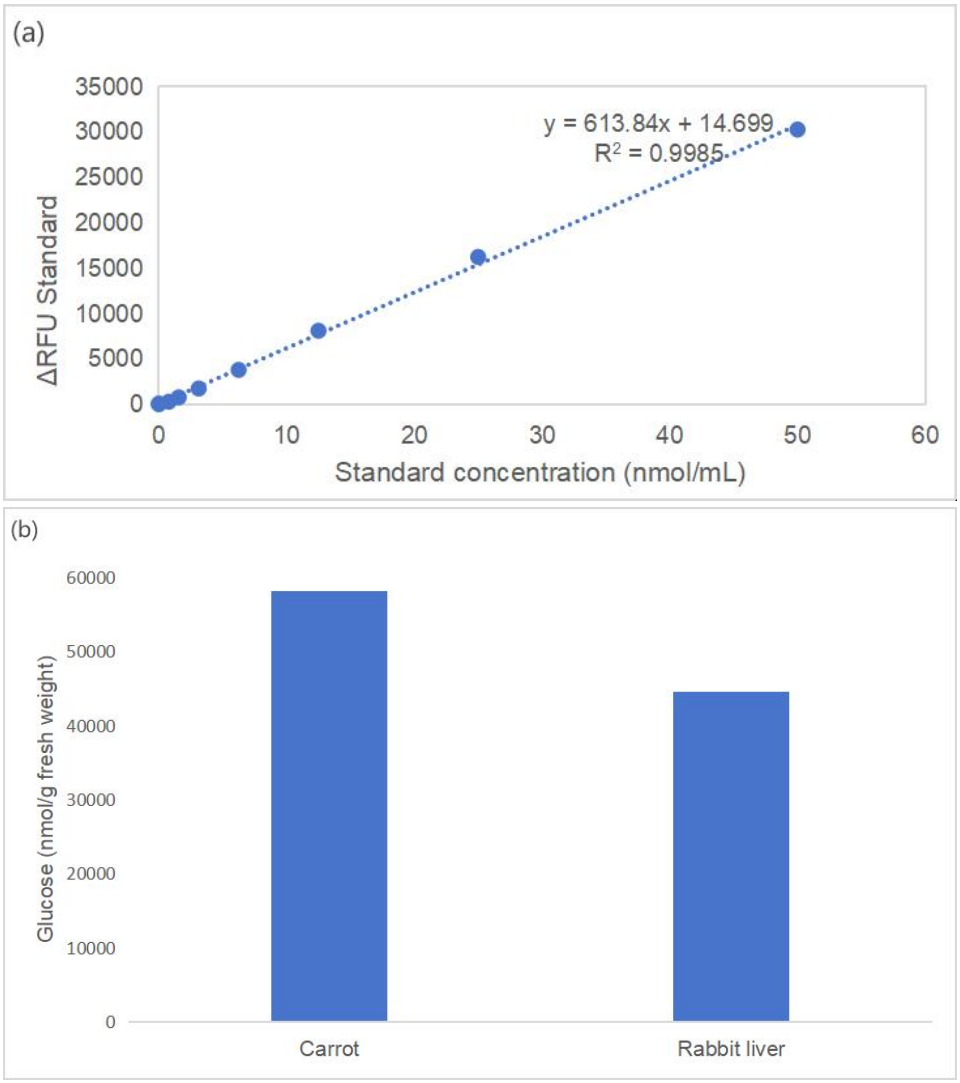
3. A new standard curve needs to be established for each test.

4. If the RFU_{Test} is low or the ΔRFU_{Test} is negative, the sample dilution ratio can be appropriately reduced or the reaction time can be extended, but it should not exceed 30 min.

5. If no value is found in the sample, it is recommended to take a fresh sample and test it again.

Typical Data

The following data are for reference only. And the experimenters need to test the samples according to their own experiments.



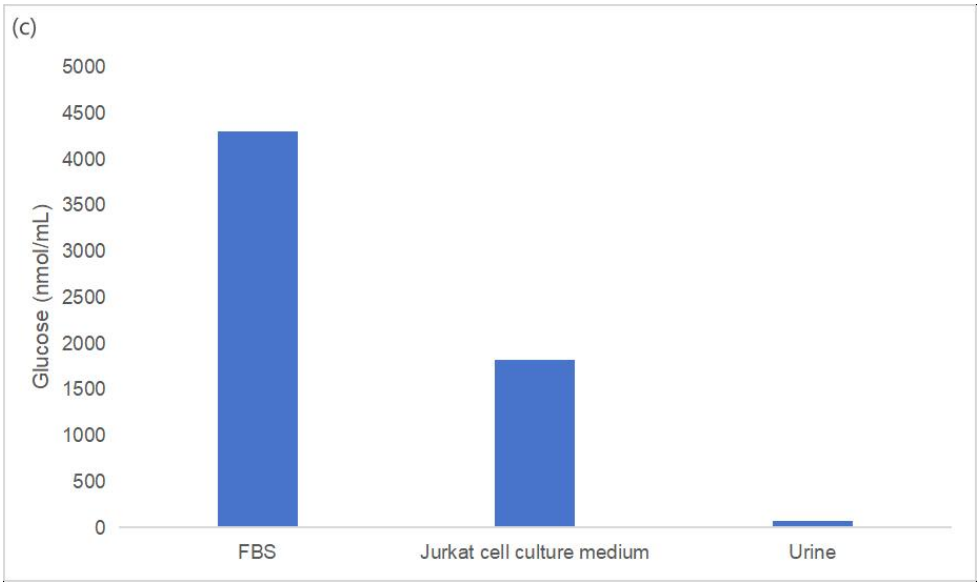


Figure 1. (a) Glucose standard curve. (b) Determination of glucose content in carrot and rabbit liver by this kit. (c) Determination of glucose content in FBS. Jurkat cell culture medium and urine by this kit.

Recommended Products

Catalog No.	Product Name
KTB9050	CheKine™ Pro Malondialdehyde (MDA) Fluorometric Assay Kit
KTB9041	CheKine™ Pro Hydrogen Peroxide (H_2O_2) Fluorometric Assay Kit
KTB9042	CheKine™ Pro Catalase (CAT) Fluorometric Activity Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.