（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-F037

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=535 nm/590 nm)

Detection range：0.1-20 μmol/L

Elabscience®Glucose (GLU) Fluorometric Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tel: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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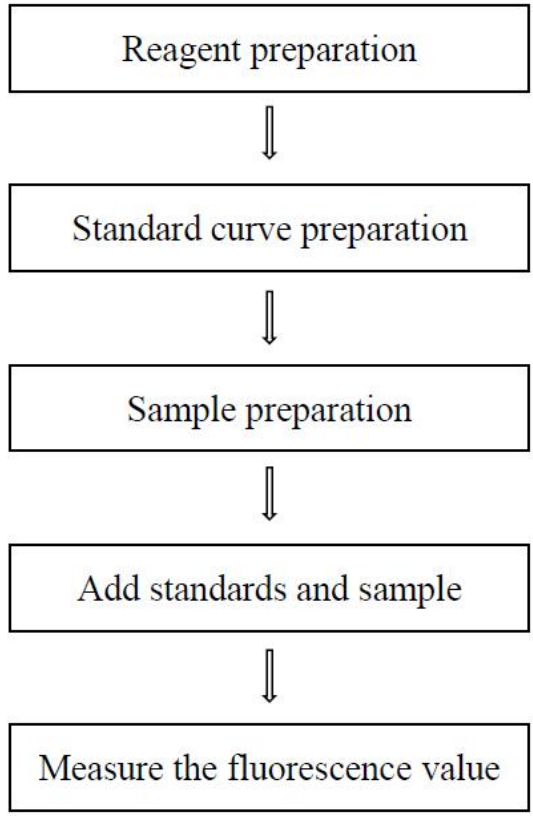
Assay summary



Intended use

This kit can be used to measure glucose (Glu) content in serum, plasma, urine, saliva, milk and cell samples.

Detection principle

Glucose oxidase can catalyze the oxidation of glucose into gluconic acid and produce hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide reacts with the non-fluorescent substance to form fluorescent substance. The glucose content can be calculated indirectly by measuring the fluorescence intensity at the excitation wavelength of 535 nm and the emission wavelength of 590 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	50 mL ×1 vial	50 mL × 2 vials	-20℃, 12 months
Reagent 2	Enzyme Reagent	Powder × 1 vial	Powder × 1 vial	-20℃, 12 months shading light
Reagent 3	Chromogenic Agent	0.125 mL ×1 vial	0.25 mL × 1 vial	-20℃, 12 months shading light
Reagent 4	5 mmol/L Standard	0.5 mL ×1 vial	0.5 mL × 1 vial	-20℃, 12 months
	Black Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Fluorescence microplate reader (Ex/Em=535 nm/590 nm), Micropipettor, Incubator, Vortex mixer, Water bath, Centrifuge

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of enzyme working solution:

Dissolve one vial of enzyme reagent with 250 μL of buffer solution, mix well to dissolve. Store at -20°C for 1 month protected from light.

③ The preparation of chromogenic agent working solution:

For each well, prepare 50 uL of chromogenic agent working solution (mix well 46 μL of buffer solution, 2 μL of enzyme working solution and 2 μL of chromogenic agent). The chromogenic agent working solution should be prepared on spot and protected from light.

④ The preparation of 50 μmol/L glucose standard：

Dilute 7 μL of 5 mmol/L Standard with 693 μL of buffer solution, mix well. Store at 2-8°C for 7 days.

⑤ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 50 μmo/L glucose standard with buffer solution to a serial concentration. The recommended dilution gradient is as follows: 0, 2, 4, 6, 8, 10, 15, 20 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**1**	**2**	**4**	**5**	**6**	**7**	**8**
50 μmol/L standard (μL)	0	20	40	80	100	120	140	160
Buffer solution (μL)	200	180	160	120	100	80	60	40

Sample preparation

① Sample preparation

Serum, plasma and other liquid sample: If the liquid sample is cloudy, centrifuge at 10000 g for 10 min at 4℃. Take the supernatant and preserve it on ice for detection. If not detected on the same day, the serum, plasma or other liquid sample can be stored at -80℃ for a month.

Cell (adherent or suspension) samples:

① Harvest the number of cells needed for each assay (initial recommendation 2×10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 2×10^6 cells in 200 μL buffer solution with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10000×g for 10 min to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
Human plasma	300-600
Human serum	300-600
Chicken serum	600-1000
Human urine	1
Human milk	400-600
Saliva	3-5

Note: The diluent is buffer solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

Avoid repeated freezing and thawing of enzyme working solution, it is recommended to aliquot the enzyme working solution into smaller quantities and store at -20℃.

Operating steps

① Standard well: add 50 μL of standards with different concentrations into the wells.
Sample well: add 50 μL of sample into the wells.

② Add 50 μL of chromogenic agent working solution and mix fully.

③ Mix fully with microplate reader for 10 s and incubate at 37℃ for 15 min with shading light.

④ Measure the fluorescence intensity of each well at the excitation wavelength of 535 nm and the emission wavelength of 590 nm.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean fluorescence value of the blank (Standard #①) from all standard readings. This is the absoluted fluorescence value.

3. Plot the standard curve by using absoluted fluorescence value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Serum (plasma) and other liquid sample

$$\text{Glu content (μmol/L)} = (\Delta F - b) \div a \times f$$

2. Cell samples

$$\text{Glu content (μmol/gprot)} = (\Delta F - b) \div a \times f \div C_{pr}$$

[Note]

ΔF: Absolute fluorescence intensity of sample ($F_{Sample} - F_{Blank}$)

f: Dilution factor of sample before test.

C_{pr}: Concentration of protein in sample, gprot/L.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	2.50	15.00	25.00
%CV	2.0	1.5	1.6

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	2.50	15.00	25.00
%CV	3.2	2.5	2.7

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 99%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (μmol/L)	3.5	7	12
Observed Conc. (μmol/L)	3.5	6.9	11.9
Recovery rate (%)	100	98	99

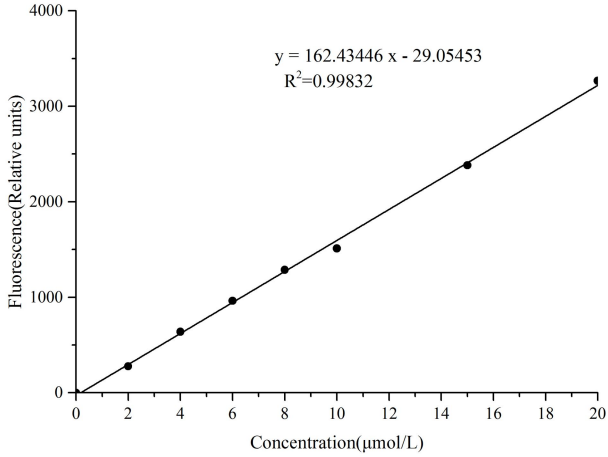
Sensitivity

The analytical sensitivity of the assay is 0.1 μmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the fluorescence value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Concentration (μmol/L)	0	2	4	6	8	10	15	20
Fluorescence value	147	427	780	1104	1446	1639	2515	3400
	148	422	791	1117	1423	1678	2546	3429
Average fluorescence value	148	424	786	1110	1434	1658	2530	3414
Absoluted fluorescence value	0	276	638	962	1286	1510	2382	3266



Appendix II Example Analysis

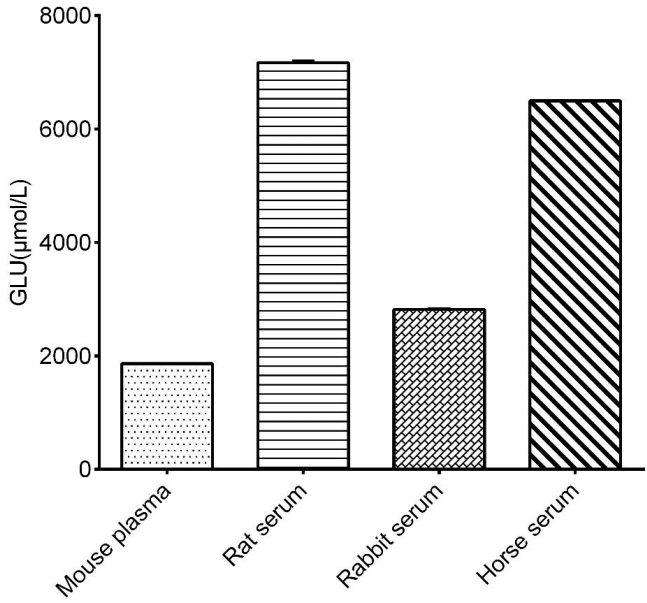
Example analysis:

For human plasma, dilute for 300 times with buffer solution, take 50 μL of diluted sample according to the instructions and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 162.43 x – 29.055, the average fluorescence value of the sample is 2741, the average fluorescence value of the blank is 80, and the calculation result is:

$$\text{GLU content (μmol/L)} = (2741 - 80 + 29.055) \div 162.43 \times 300 = 4968.40 \text{ μmol/L}$$

Detect mouse serum (dilute for 200 times), rat serum (dilute for 600 times), rabbit serum (dilute for 200 times) and horse serum (dilute for 600 times) according to the protocol, the result is as follows:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.