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

Catalog No：E-BC-K234-M

Specification：48T(32 samples)/96T(80 samples)/96T×5(464 samples)

Measuring instrument：Microplate reader（500-510 nm）

Detection range：0.04-30 mmol/L

Elabscience® Glucose (Glu) Colorimetric Assay Kit (GOD-POD Method)

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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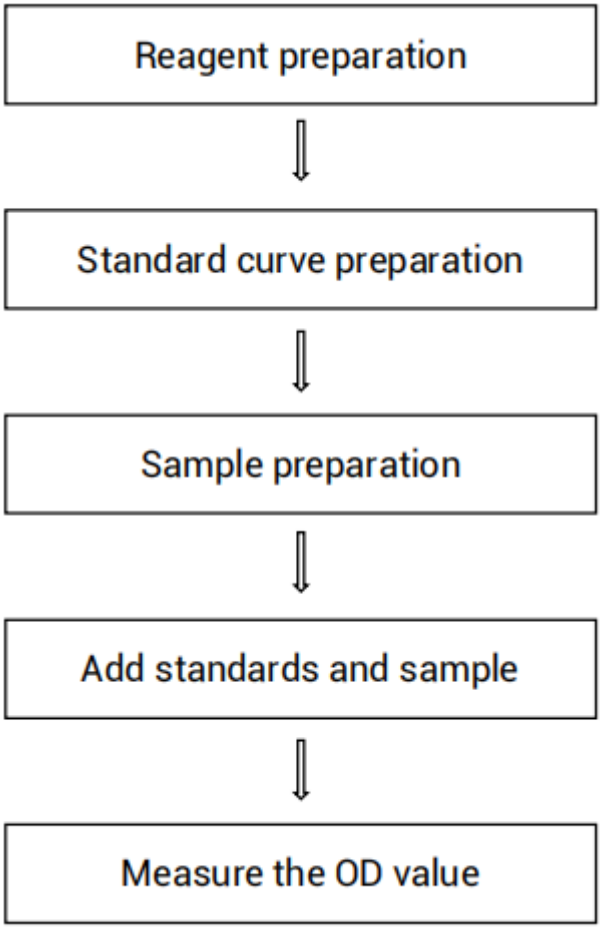
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Assay summary



Intended use

This kit can be used to measure the Glucose (Glu) content in whole blood, serum, plasma and tissue samples.

Detection principle

Glucose oxidase can catalyze the oxidation of glucose to gluconic acid to produce hydrogen peroxide. In the presence of chromogenic oxygen receptors, peroxidase catalyzes hydrogen peroxide and oxidizes pigment sources to form colored substances. Measure the OD value at 505 nm and glucose content can be calculated indirectly.

Kit components & storage

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Size 3 (96 T×5)	Storage
Reagent 1	Phenol Solution	10 mL×1 vial	20 mL×1 vial	50 mL×2 vials	2-8°C, 12 months shading light
Reagent 2	Enzyme Solution	10 mL×1 vial	20 mL×1 vial	50 mL×2 vials	2-8°C, 12 months shading light
Reagent 3	50 mmol/L Glucose Standard	1.2 mL×1 vial	1.2 mL×1 vial	6 mL×1 vial	2-8°C, 12 months
	Microplate	48 wells	96 wells	96 wells×5	No requirement
	Plate Sealer	2 pieces		7 pieces	
	Sample Layout Sheet	1 piece		7 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:

Microplate reader (500-510 nm), Vortex mixer, Micropipettor, Incubator

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② The preparation of enzyme working solution：

For each well, prepare 300 μL of enzyme working solution (mix well 150 μL of phenol solution and 150 μL of enzyme solution). Store at 2-8°C for 24 h protected from light.

③ The preparation of control working solution：

For each well, prepare 300 μL of control working solution (mix well 150 μL of normal saline and 150 μL of enzyme solution). Store at 2-8°C for 24 h protected from light.

④ The preparation of standard curve：

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

Dilute 50 mmol/L glucose standard with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 2, 5, 10, 15, 20, 25, 30 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**2**	**5**	**10**	**15**	**20**	**25**	**30**
50 mmol/L glucose standard(μL)	0	4	10	20	30	40	50	60
Double distilled water (μL)	100	96	90	80	70	60	50	40

Sample preparation

① Sample preparation

Serum, plasma and whole blood: detect directly. If not detected on the same day, the serum or plasma can be stored at -80°C for a month.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold normal saline (0.9% NaCl).

③ Homogenize 20 mg tissue in 180 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 min at 4°C 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 serum	1
Mouse serum	1
Rat serum	1
Human plasma	1

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please do pretest to confirm the dilution factor

The key points of the assay

① Control wells must be set for whole blood samples as well as hemolyzed serum and plasma samples. Control wells are not required for normal serum, plasma, and tissue samples.

② Enzyme solution. should be aliquoted before use to avoid contamination.

Operating steps

① Standard well: Take 3 μL of standard solution with different concentration to the wells.

Sample well: Take 3 μL of sample to the wells.

Control well: Take 3 μL of sample to the wells.

② Add 300 μL of enzyme working solution into the standard and sample well.

Add 300 μL of control working solution into the control well.

③ Cover the plate sealer and incubate at 37 °C for 15 min.

④ Measure the OD value of each well with microplate reader at 505 nm.

Note: Set control wells for whole blood, hemolysis serum and plasma samples, but not for normal serum, plasma and tissue samples.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

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

3. Plot the standard curve by using absoluted OD 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. Normal serum (plasma) sample:

$$\text{Glu content (mmol/L)} = (\Delta A_{505} - b) \div a \times f$$

2. Whole blood and hemolysis sample:

$$\text{Glu content (mmol/L)} = (\Delta A' - b) \div a \times f$$

3. Tissue sample:

$$\text{Glu content (mmol/gprot)} = (\Delta A_{505} - b) \div a \times f \div C_{pr}$$

[Note]

ΔA_{505}: $OD_{Sample} - OD_{Blank}$.

$\Delta A'$: $OD_{Sample} - OD_{Control}$.

f: Dilution factor of sample before tested.

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 (mmol/L)	1.50	13.40	25.50
%CV	2.4	2.1	1.2

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 (mmol/L)	1.50	13.40	25.50
%CV	2.5	1.7	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 100%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	3.6	11.5	24.5
Observed Conc. (mmol/L)	3.6	11.4	24.5
recovery rate(%)	101	99	100

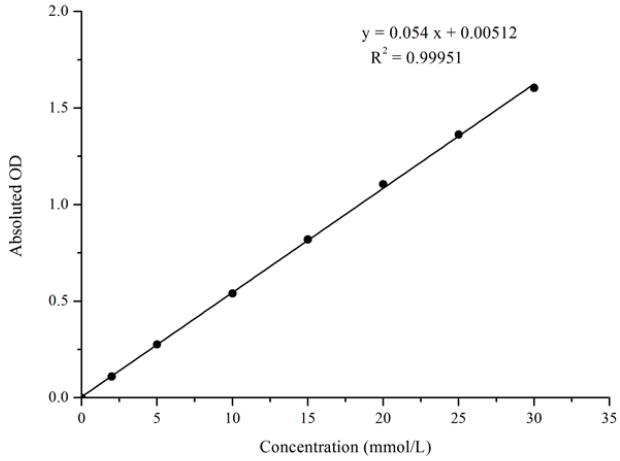
Sensitivity

The analytical sensitivity of the assay is 0.04 mmol/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 OD 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 (mmol/L)	0	2	5	10	15	20	25	30
Average OD	0.043	0.152	0.318	0.583	0.863	1.148	1.406	1.647
Absoluted OD	0	0.110	0.276	0.540	0.820	1.106	1.363	1.604



Appendix Π Example Analysis

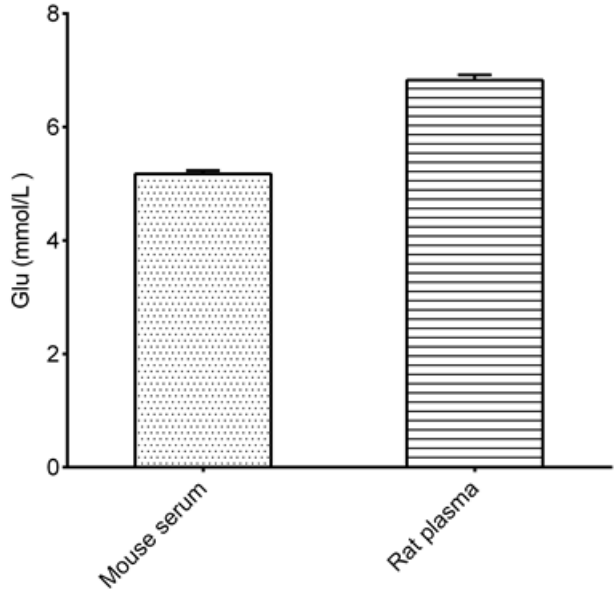
Example analysis：

Take 3 μL of mouse serum, carry the assay according to the operation steps. The results are as follows:

standard curve: y = 0.054 x + 0.00512, the average OD value of the sample is 0.327, the average OD value of the blank is 0.043, and the calculation result is:

Glu content (mmol/L) = (0.327 - 0.043 - 0.00512) ÷ 0.054 = 5.17 mmol/L

Detect mouse serum, rat plasma, according to the protocol, the result is as follows：



Appendix III Publications

1. Liu L Z, Wang B, Zhang R, et al. The activated CD36-Src axis promotes lung adenocarcinoma cell proliferation and actin remodeling-involved metastasis in high-fat environment[J]. Cell Death & Disease, 2023, 14(8): 548.
2. Zhao Z, Yang R, Li M, et al. Effects of ambient temperatures between 5 and 35° C on energy balance, body mass and body composition in mice[J]. Molecular Metabolism, 2022, 64: 101551.
3. Albrahim T. Lycopene modulates oxidative stress and inflammation in hypercholesterolemic rats[J]. Pharmaceuticals, 2022, 15(11): 1420.
4. Paul S, Pallavi A, Gandasi N R. Exploring the potential of pheophorbide A, a chlorophyll-derived compound in modulating GLUT for maintaining glucose homeostasis[J]. Frontiers in Endocrinology, 2024, 15: 1330058.
5. Chen W, Li Y, Zhong J, et al. circ-PRKCI targets miR-1294 and miR-186-5p by downregulating FOXK1 expression to suppress glycolysis in hepatocellular carcinoma[J]. Molecular Medicine Reports, 2021, 23(6): 464.
6. Dede A F Ü, Arslanyolu M. The in vivo Tetrahymena thermophila extracellular glucose drop assay for characterization of mammalian insulin activity[J]. European Journal of Protistology, 2021, 79: 125803.

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.