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

Catalog No：E-BC-K234-S

Specification：50 Assays(48 samples)/100 Assays (96 samples)

Measuring instrument：Spectrophotometer（505 nm）

Detection range：0.05-30 mmol/L

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

This manual must be read attentively and completely before using this product.

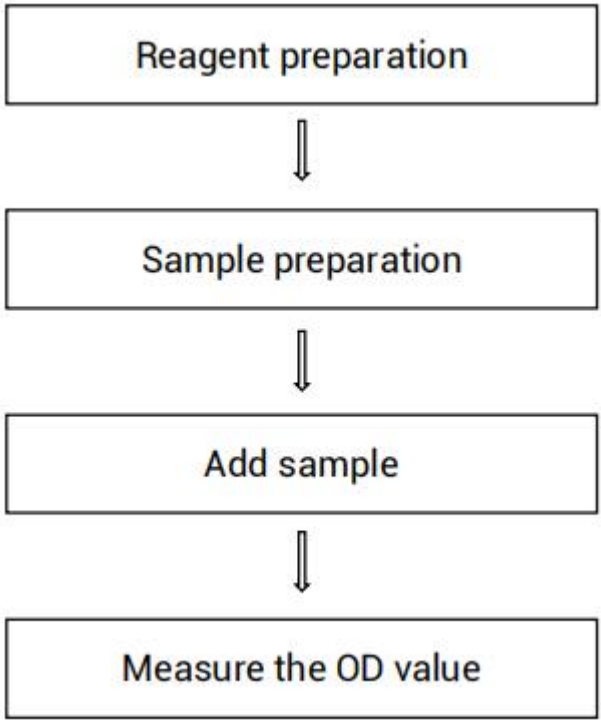
If you have any problem, please contact our Technical Service Center for help：

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.

Table of contents

Assay summary



Intended use

This kit can be used to measure the glucose (Glu) content in 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 (50 assays)	Size 2 (100 assays)	Storage
Reagent 1	Phenol Solution	60 mL × 1 vial	60 mL × 2 vials	2-8°C, 12 months shading light
Reagent 2	Enzyme Solution	60 mL × 1 vial	60 mL × 2 vials	2-8°C, 12 months shading light
Reagent 3	5 mmol/L Glucose Standard	1 mL × 1 vial	1 mL × 1 vial	2-8°C, 12 months

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:

Spectrophotometer (505 nm), Vortex mixer, Magnetic Stirrers, Micropipettor, 37°C 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 tube, prepare 2000 μL of enzyme working solution (mix well 1000 μL of phenol solution and 1000 μL of enzyme solution). The enzyme working solution should be prepared on spot. Store at 2-8°C for 24 h protected from light.

Sample preparation

① Sample preparation

Serum and plasma: 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
Rat/ Human plasma	1
Mouse/Human serum	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 Separate serum or plasma from red blood cell immediately after blood collection to avoid the glycolysis

② Serum and plasma samples must be clarified.

Operating steps

① Standard tube: add 2000 μL of enzyme working solution into the 5 mL EP tube.

Sample tube: add 2000 μL of enzyme working solution into the 5 mL EP tube.

Blank tube: add 2000 μL of enzyme working solution into the 5 mL EP tube.

② Blank tube: Add 20 μL of double distilled water.

Standard tube: Add 20 μL of 5 mmol/L Glucose Standard.

Sample tube: Add 20 μL of sample.

③ Mix well and incubate at 37°C for 25 min.

④ Set to zero with double distilled water and measure the OD value of each tube with 1 cm optical path cuvette at 505 nm.

Calculation

1. Serum (plasma) sample:

$$\text{Glu content (mmol/L)} = \frac{\Delta A_1}{\Delta A_2} \times c \times f$$

2. Tissue sample:

$$\text{Glu content (mmol/gprot)} = \frac{\Delta A_1}{\Delta A_2} \times c \times f \div C_{pr}$$

[Note]

ΔA_1: $OD_{Sample} - OD_{Blank}$.

ΔA_2: $OD_{Standard} - OD_{Blank}$.

c: Concentration of standard (5 mmol/L).

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)	2.60	18.40	25.70
%CV	1.5	1.3	0.8

Inter-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	2.60	18.40	25.70
%CV	1.0	1.4	1.5

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 101%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (mmol/L)	4.5	15	22
Observed Conc. (mmol/L)	4.5	15.5	22.2
recovery rate(%)	99	103	101

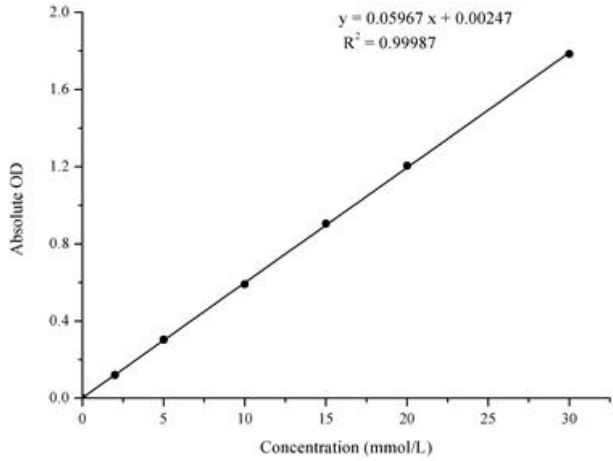
Sensitivity

The analytical sensitivity of the assay is 0.05 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	30
Average OD	0.007	0.128	0.310	0.598	0.912	1.212	1.792
Absoluted OD	0	0.121	0.303	0.591	0.905	1.205	1.785



Appendix Π Example Analysis

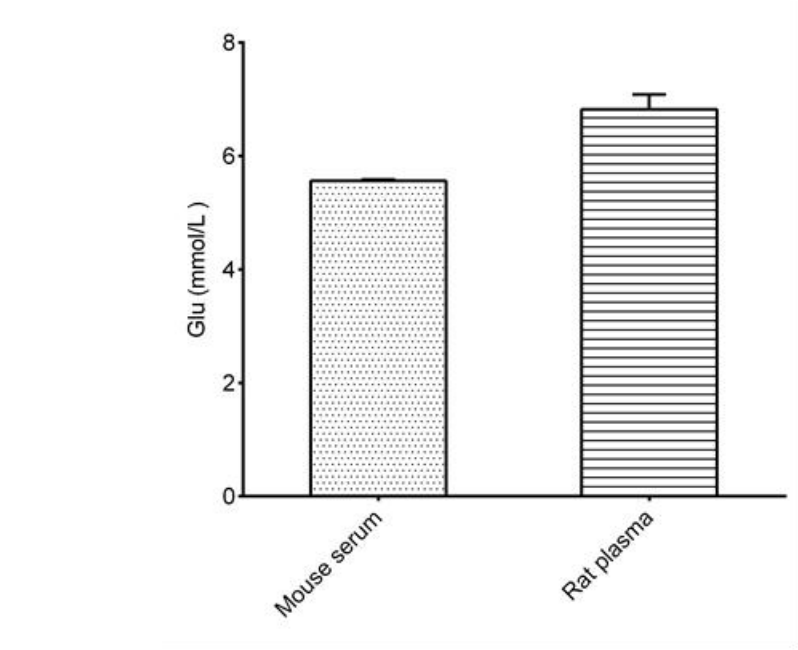
Example analysis：

Take 0.02 mL of mouse serum and carry the assay according to the operation steps. The results are as follows:

the average OD value of the standard is 0.308, the average OD value of the sample is 0.343, the average OD value of the blank well is 0.009, and the calculation result is:

$$\text{Glu content (mmol/L)} = \frac{0.343 - 0.009}{0.308 - 0.009} \times 5 = 5.56 \text{ mmol/L}$$

Detect mouse serum, rat plasma, 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.