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

Catalog Number: K085

Size: 48T(32S)/ 96T(80S)

Detection instrument: Microplate reader(500-510 nm)

Species: Universal

Application: Quantitative detection of the glucose (Glu) content in Whole blood, serum, plasma and tissue samples.

Storage Conditions: 2-8°C for 6 months

Shelf life: See kit label

Note: For research use only.

Reagent Components:

Item	Component	Size 1 48T	Size2 96T	Storage
Reagent 1	Phenol Solution	10 mLx1 vial	20 mLx1 vial	2-8°C, 6 months shading light
Reagent 2	Enzyme Solution	10 mLx1 vial	20 mLx1 vial	2-8°C, 6 months shading light
Reagent 3	50 mmol/L Glucose Standard	1.2 mLx1 vial	1.2 mLx1 vial	2-8°C, 6 months

Assay 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.

Additional Materials Required:

1. Microplate reader (500-510 nm)
2. 96-well plate
3. Precision Micropipettor and clean disposable tips
4. Clean EP tubes
5. Normal saline (0.9% NaCl)，Double distilled water
6. Benchtop centrifuge
7. Constant temperature water bath/incubator

Sample Preparation:

Serum and plasma: detect directly.

whole blood: Take fresh blood and add it into the tube containing anticoagulant (heparin as anticoagulant, heparin concentration: 10-12.5 IU/mL blood), mix it upside down, take 0.1 mL and add 0.4 mL of double distilled water, mix it fully for 1 min, stand for 15 min. In this way, 5 times dissolved blood is prepared, which is clarified and transparent under light observation to be measured.

Before the formal test, 2-3 samples with significant differences should be selected and diluted into different concentrations for pre-test.

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

Sample type	Dilution factor	Sample type	Dilution factor
Human serum	No Dilution	Human plasma	No Dilution
Rat serum	No Dilution	Mouse serum	No Dilution

Note: The diluent is normal saline (0.9% NaCl).

Reagent preparation:

1. Equilibrate all reagents to room temperature before use.

2. The preparation of enzyme working solution:

Mix Reagent 1 and Reagent 2 at the volume ratio of 1:1 before use, and store at 2-8° C away from light for 24 h.

3. The preparation of control working solution:

Mix normal saline and Reagent 2 at the volume ratio of 1:1 before use, and store at 2-8° C away from light for 24 h.

4. The preparation of standards at different concentrations

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 (μ L)	0	4	10	20	30	40	50	60
Double distilled water (μ L)	100	96	90	80	70	60	50	40

Assay Procedure:

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. 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.

Operation table:

	Standard well	Sample well	Control well
glucose standard with different concentration(μL)	3		
Sample to be tested(μL)		3	3
enzyme working solution(μL)	300	300	
control working solution(μL)			300
Incubate at 37 ° C for 15 min. Measure the OD value of each well with microplate reader at 505 nm.			

Calculation:

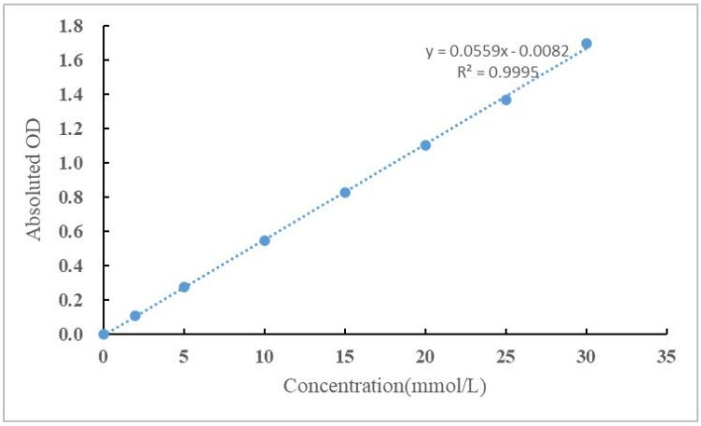
the standard curve: **y=ax+b**

Normal serum (plasma) sample: Glu content (mmol/L) = (ΔA505 - b) ÷ a × f

Whole blood and hemolysis sample: Glu content (mmol/L) = (ΔA' - b) ÷ a × f

Note: y：$OD_{Standard} - OD_{Blank}$; x：Concentration of standard; a：The slope of the standard curve; b：The intercept of the standard curve; ΔA505：$OD_{Sample} - OD_{Blank}$; ΔA'：$OD_{Sample} - OD_{Control}$; f：Dilution factor of sample before tested.

Standard curve: (The standard curve is provided as below for reference only.)



Note:

1. This assay kit is for Research Use Only. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments. Protection methods must be taken by wearing lab coat and latex gloves.

2. The detection range of the kit is not equivalent to the concentration range of the substance to be measured in the sample. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample. It is recommended to take a pre-test if your sample is not listed in the instruction book.

3. The experimental results are closely related to the situation of reagents, operations, environment and so on. Fine Test 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.