PRODUCT INFORMATION & MANUAL

Glucose Assay Kit (Colorimetric)

NBP3-24489

For research use only.

Not for diagnostic or therapeutic procedures.

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Glucose Assay Kit (Colorimetric)

(GOD-POD Method)

Catalog No: NBP3-24489

Method: Colorimetric method

Specification: 100 Assays (Can detect 96 samples without duplication)

Instrument: Spectrophotometer

Sensitivity: 0.05 mmol/L

Detection range: 0.05-30 mmol/L

Average intra-assay CV(%): 1.2

Average inter-assay CV(%): 1.3

Average recovery rate(%): 101

_. This kit is for research use only.

_. Instructions should be followed strictly, changes of operation may result in unreliable results.

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

General information

• Intended use

This kit can be used to measure Glucose (Glu) content in serum, plasma samples.

• Background

It is very important for diagnosis of hyperglycemia to accurate determination of glucose. Usually, there is also a variety of inhibition test and determination of glucose tolerance test at the same time with glucose measuring during finding the cause of these conditions. Glucose level increases seen in diabetes mellitus, glucose intake, cushing syndrome and cerebrovascular accident. Glucose content decreases seen in insulinoma, insulin overdose and congenital carbohydrate metabolism disorder.

• 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	Specification	Storage
Reagent1	Phenol Solution	60 ml x 2 vials	2-8°C , 12 months, shading light
Reagent2	Enzyme Solution	60 ml x 2 vials	2-8°C , 12 months, shading light
Reagent3	5 mmol/l Glucose Standard	1 ml x 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.			

• Materials prepared by users

Instruments

Spectrophotometer (505 nm), Vortex mixer, Micropipettor, Incubator

Reagents

Double distilled water, Normal saline (0.9% NaCl)

A. Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

A Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

• 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.

Pre-assay preparation

• Reagent preparation

The preparation of enzyme working solution:

Mix the reagent 1 and reagent 2 at a ratio of 1:1. Prepare the fresh solution before use. It can be stored at 2-8°C for 24 hours with shading light.

• Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

• Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.05-30 mmol/L).

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

Sample type	Dilution factor
Rat plasma	1
Mouse serum	1
Human serum	1
Human plasma	1

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

Assay protocol

• Detailed operating steps

1. Standard tube: Take 2000 μl of enzyme working solution into the 5 ml EP tube.
   Sample tube: Take 2000 μl of enzyme working solution into the 5 ml EP tube.
   Blank tube: Take 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 fully 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. (After color development, the color is stable for at 2h.)

• Summary operation table

	Blank tube	Standard tube	Sample tube
Enzyme working solution (μl)	2000	2000	2000
Double distilled water (μl)	20		
5 mmol/l Glucose standard (μl)		20	
Sample (μl)			20
Mix fully, incubate in 37°C water bath for 25 min. Set to zero with double distilled water and measure the OD values of each tube at 505 nm.			

Calculation

Serum (plasma):

$$\text{Glu content (mmol/L)} = \frac{!::,.A1}{!::,.A2} \times c \times f$$

Note:

M1: ODsample- ODBlank

ΔA_2: $OD_{Standard} - OD_{Blank}$

C: Concentration of standard (5 mmol/L)

f: Dilution factor of sample before test

Appendix I Data

Example analysis

Take 0.02 ml of mouse serum and carry the assay according to the operation table.

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, the calculation result is:

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

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

.. serum

Collect fresh blood and stand at 25°e for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4°e . Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at *-B0°e* for a month.

_.Plasma

Take fresh blood into the tube which has anticoagulant (heparin is used as anticoagulant), centrifuge at 700-1000 g for 10 min at 4°e. Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection. If not detected on the same day, the plasma can be stored at *-B0°e* for a month.

_. Notes for sample

1. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

2. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Appendix III References

1. Jones C E, Koshibu K, Decambre M, et al. The Kidney's role in glucose balance following partial hepatectomy[J]. Journal of Surgical Research, 1998, 79(2): 136-140.
2. Martin C. The physiology of amylin and insulin: maintaining the balance between glucose secretion and glucose uptake[J]. Diabetes Educ, 2006, 32 Suppl 3: 101S-104S.