PRODUCT INFORMATION & MANUAL

Glucose Assay Kit (Colorimetric)

NBP3-24488

For research use only.

Not for diagnostic or therapeutic procedures.

www.novusbio.com - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 - technical@novusbio.com

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

Catalog No: NBP3-24488

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Instrument: Microplate reader

Sensitivity: 0.04 mmol/L

Detection range: 0.04-30 mmol/L

Average intra-assay CV(%): 1.9

Average inter-assay CV(%): 2.3

Average recovery rate(%): 100

_. 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 whole blood, 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	20 ml x 1 vial	2-8°C , 12 months, shading light
Reagent2	Enzyme Solution	20 ml x 1 vial	2-8°C , 12 months, shading light
Reagent3	50 mmol/l Glucose Standard	1.2 ml x 1 vial	2-8°C , 12 months
	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.			

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

2. To avoid contamination, do not put the pipette directly into the reagent bottle when using reagent 2.

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.

The Preparation of control working solution:

Mix the normal saline 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 11.

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 (0.04-30 mmol/L).

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) .

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.

• Detailed operating steps

The preparation of standard curve

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:

Number	Standard concentrations (mmol/L)	50 mmol/LStandard(μL)	Double distilled water (μL)
A	0	0	100
B	2	4	96
C	5	10	90
D	10	20	80
E	15	30	70
F	20	40	60
G	25	50	50
H	30	60	40

The measurement of samples

(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 and plasma.

Summary operation table

	Standard well	Sample well	Control well
Standard solutions with different concentration (μL)	3		
Sample (μL)		3	3
Enzyme working solution (μL)	300	300	
Control working solution (μL)			300
Cover the plate sealer and incubate at 37°C for 15 min. Measure the OD value at 505 nm.			

Calculation

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. If the sample have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. The actual concentration is the calculated concentration multiplied by dilution factor.

The standard curve is: y= ax+ b.

1. Normal serum (plasma):

$$\text{Glu content (mmol/L)} = (\text{liA}_{505} - b) + a \times f$$

2. Whole blood and hemolysis samples:

$$\text{Glu content (mmol/L)} = (\text{liA}' - b) + a \times f$$

Note:

y: ODstandard - ODBlank (ODBlank is the OD value when the standard concentration is 0)

x: The concentration of standard

a: The slope of standard curve

b: The intercept of standard curve

fiAsos: ODsample - ODBlank

fiA' : ODsample - QDcontrol

f: Dilution factor of sample before test

Appendix I Data

• Example analysis

Take 3 μL of mouse serum, carry the assay according to the operation table.

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, the calculation result is:

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

_.Whole blood

Take fresh blood to the tube containing heparin anticoagulant and mix it upside and down, then take 0.1 ml of the whole blood and add 0.4 ml of pre-cooled double distilled water. Mix fully for 1 min and stand for 15 min until the prepared hemolysis is transparent when observing under light.

_. 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.