PRODUCT INFORMATION & MANUAL

Glucose 6 Phosphate Assay Kit (Colorimetric)

NBP3-24492

For research use only.

Not for diagnostic or therapeutic procedures.

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Glucose 6 Phosphate Assay Kit
(Colorimetric)

Method: Colorimetric method

Specification: 96T (Can detect 40 samples without duplication)

Measuring instrument: Microplate reader

Sensitivity: 5.6 μmol/L

Detection range: 5.6-500 μmol/L

Average intra-assay CV (%): 2.1

Average inter-assay CV (%): 4.3

Average recovery rate (%): 95

▲ 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-6-phosphate (G6P) content in serum, plasma and animal tissue samples.

Background

Glucose-6-phosphate (G6P) is a molecule generated by phosphorylation of hydroxyl groups on the sixth carbon of glucose under the catalysis of hexokinase. It is a common small molecule of sugar metabolism in cells and participates in biochemical pathways such as glycolysis and pentose phosphate pathway. In the first reaction of glycolysis, glucose is catalyzed by hexokinase to produce glucose-6-phosphate, which is then catalyzed by phosphoglucose isomerase to form fructose-6-phosphate to continue the other steps of glycolysis: In the pentose phosphate pathway, glucose-6-phosphate is the first substrate, and this process is also the main way to generate NADPH. In addition to these two metabolic pathways, glucose-6-phosphate can also be converted into glycogen or starch and stored.

Detection principle

Under the presence of glucose-6-phosphate dehydrogenase, glucose-6-phosphoric acid is oxidized to gluconolactone-6-phosphate (6-PG), NADP+ is reduced to NADPH. Under the action of electron coupling reagent 1-MPMS, NADPH reduce WST-8 to form orange formazan, which has the maximum absorption peak at about 450 nm. Formazan generated in the reaction system is proportional to the content of total G6P in the sample.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Extracting Solution	60 mL × 2 vials	-20℃ , 12 months
Reagent 2	Buffer Solution	5 mL × 1 vial	-20℃ , 12 months
Reagent 3	Chromogenic Agent	1.5 mL × 2 vials	-20℃ , 12 months, shading light
Reagent 4	Enzyme Agent	Powder × 1 vial	-20℃ , 12 months
Reagent 5	10 mmol/L G6P Standard	0.5 mL × 1 vial	-20℃ , 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

Microplate reader (450 nm), Micropipettor, Centrifuge, Incubator

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL, 10 mL)

Reagents

Double distilled water

▲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.

▲Precautions

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

▲The key points of the assay

There should be no bubbles in the wells of the microplate when measuring the OD value.

Pre-assay preparation

▲Reagent preparation

1. Bring all reagents to room temperature before use.
2. Preparation of 1 mmol/L standard solution:

   Mix the reagent 5 and reagent 1 at a ratio of 1:9 fully. Prepare the fresh solution before use.
3. Preparation of reagent 4 working solution:

   Dissolve reagent 4 with 1.8 mL of reagent 2. Prepare the fresh solution before use and the prepared solution can be stored with aliquot at -20℃ for 7 days.
4. Preparation of control working solution:

   Mix the reagent 2 and reagent 3 at a ratio of 1:1fully. Prepare the fresh solution before use and store it with shading light.

   Preparation of sample working solution:

   Mix the reagent 3 and reagent 4 working solution at a ratio of 1:1 fully. Prepare the fresh solution before use and store it with shading light.

▲Sample preparation

1. Serum (Plasma): Detect the sample directly.
2. Tissue sample: Weigh the tissue accurately. Add reagent 1 in a weight (g): volume (mL) ratio of 1: 9, homogenize mechanically in ice water bath to break cells fully. Then centrifuge at 10000 g for 10 min at 4℃ and collect the supernatant for measurement. Meanwhile, determine the protein concentration of supernatant.

▲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 (5.6-500 μmol/L).

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

Sample type	Dilution factor
Human plasma	1
Mouse serum	2-3
Rat plasma	1
Porcine serum	1
10% Rat spleen tissue homogenate	2-3
10% Rat heart tissue homogenate	2-3
10% Rat liver tissue homogenate	2-3
10% Mouse lung tissue homogenate	1

Note: The diluent is reagent 1.

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S1'	S9	S9'	S17	S17'	S25	S25'	S33	S33'
B	B	B	S2	S2'	S10	S10'	S18	S18'	S26	S26'	S34	S34'
C	C	C	S3	S3'	S11	S11'	S19	S19'	S27	S27'	S35	S35'
D	D	D	S4	S4'	S12	S12'	S20	S20'	S28	S28'	S36	S36'
E	E	E	S5	S5'	S13	S13'	S21	S21'	S29	S29'	S37	S37'
F	F	F	S6	S6'	S14	S14'	S22	S22'	S30	S30'	S38	S38'
G	G	G	S7	S7'	S15	S15'	S23	S23'	S31	S31'	S39	S39'
H	H	H	S8	S8'	S16	S16'	S24	S24'	S32	S32'	S40	S40'

Note: A-H, standard wells; S1-S40, sample wells; S1'- S40', control wells.

▲Detailed operation steps

The preparation of standard curve

Dilute 1 mmol/L standard solution with reagent 1 to a serial concentration. The recommended dilution gradient is as follows: 0, 50, 100, 200, 300, 350, 400, 500 μmol/L. Reference is as follows:

Number	Standard concentrations (μmol/L)	1 mmol/L standard solution (μL)	Reagent 1 (μL)
A	0	0	200
B	50	10	190
C	100	20	180
D	200	40	160
E	300	60	140
F	350	70	130
G	400	80	120
H	500	100	100

The measurement of samples

1. Standard well: add 50 μL of standard solution with different concentrations into the corresponding wells.
   Sample well: add 50 μL of sample into the sample wells.
   Control well: add 50 μL of sample into the control wells.
2. Add 50 μL of sample working solution into the sample wells and standard wells.
   Add 50 μL of control working solution into the control wells
3. Mix fully for 5 s with microplate reader and incubate at 37℃ for 10 min.
4. Measure the OD values of each well at 450 nm with microplate reader. The OD values of sample well recorded as A_2, the OD values of control well recorded as A_1, then $\Delta A = A_2 - A_1$.

▲Summary operation table

	Standard well	Sample well	Control well
Standards with different concentrations (μL)	50		
Sample (μL)		50	50
Sample working solution (μL)	50	50	
Conrtol working solution (μL)			50
Mix fully for 5 s and incubate at 37℃ for 10 min. Measure the OD values of each well. The OD values of sample well recorded as A_2, the OD values of control well recorded as A_1, then $\Delta A = A_2 - A_1$.			

▲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.
The standard curve is: y= ax + b.

1. Serum (plasma) sample

$$\text{G6P content (μmol/L)} = (\Delta A - b) \div a \times f$$

2. Tissue sample

$$\text{G6P content (μmol/gprot)} = (\Delta A - b) \div a \div C_{pr} \times f$$

Note:

y: $OD_{Standard} - OD_{Blank}$ (OD_{Blank} 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;

ΔA: $\Delta A = A_2 - A_1$;

f: Dilution factor of sample before test;

C_{pr}: Concentration of protein in sample, gprot/L.

Appendix I Performance characteristics

▲Example analysis

For rat plasma, take 50 μL of sample into the sample wells and standard wells and carry the assay according to the operation table. The results are as follows: Standard curve: $y = 0.0027 x – 0.0207$, the average OD value of the control is 0.370, recorded as A_1, the average OD value of the sample is 0.421, recorded as A_2, then $\Delta A = A_2 – A_1 = 0.051$, and the calculation result is:

G6P content (μmol/L)=(0.051+0.0207)÷0.0027=26.56μmol/L