PRODUCT INFORMATION & MANUAL

Glucose 6 Phosphate Dehydrogenase Activity Assay Kit (Colorimetric)

NBP3-24493

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 6 Phosphate Dehydrogenase Activity Assay Kit (Colorimetric)

Catalog No: NBP3-24493

Method: Colorimetric method

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

Measuring instrument: Microplate reader

Sensitivity: 0.01 U/L

Detection range: 0.01-50 U/L

Average intra-assay CV (%): 2.1

Average inter-assay CV (%): 5.8

- ▲ 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 Dehydrogenase (G-6-PD) activity in serum, plasma and animal tissue samples.

▲Background

Glucose-6-phosphate dehydrogenase (G-6-PD) is a cytosolic enzyme in the pentose phosphate pathway, it can catalyze the conversion of glucose-6-phosphate to internal lipids of 6-phosphate gluconate, which is the first step of the pentose phosphate pathway and the rate-limiting step of the pathway. Pentose-phophate pathway can provide energy to cells (such as red blood cells) by maintaining the level of coenzyme nicotinamide adenine dinucleotide phosphate (NADPH). The pentose phosphate pathway is vital for the generation of NADPH and pentose, so the lack of G-6-PD which can't generate NADPH leads to some diseases, such as neonatal jaundice, non-immune hemolytic anemia.

▲Detection principle

Under the presence of G-6-PD, glucose-6-phosphoric acid is oxidized to 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 activity of G-6-PD in the sample.

▲Kit components & Storage

Item	Component	Specification	Storage
Reagent 1	Extracting Solution	50 mL × 2 vials	-20℃ , 12 months
Reagent 2	Substrate	1.5 mL × 2 vials	-20℃ , 12 months
Reagent 3	Chromogenic Agent	1.5 mL × 2 vials	-20℃ , 12 months, shading light
Reagent 4	Buffer Solution	4 mL × 1 vial	-20℃ , 12 months
Reagent 5	Standard	Powder × 1 vial	-20℃ , 12 months, shading light
	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), Pipettor, Water bath, Centrifuge

⚠ 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

1. When the reagent 1 is taken, it should be poured out part of it to avoid contamination.
2. Prevent the formulation of bubbles when the supernatant is transferred into the microplate.

Pre-assay preparation

▲Reagent preparation

1. Bring the reagent 1, reagent 3, reagent 4, reagent 5 to room temperature before use.
2. Preparation of sample working solution:
   Mix the reagent 2 and reagent 3 at a ratio of 1:1 fully. Prepare the fresh solution before use.
3. Preparation of control working solution:
   Mix the reagent 3 and reagent 4 at a ratio of 1:1 fully. Prepare the fresh solution before use.
4. Preparation of 5 mmol/L standard solution:
   Dissolve reagent 5 with 0.72 mL of double distilled water and mix fully, prepare the fresh needed amount before use.
5. Preparation of 500 μmol/L standard solution:
   Mix 5 mmol/L standard and double distilled water at a ratio of 1:9 fully. Prepare the fresh solution before use.

▲Sample preparation

▲Serum (Plasma): Detect directly

10% Tissue homogenate: Accurately weigh the tissue sample, add reagent 1 according to the ratio of Weight (g): Volume (mL) =1:9. Mechanical homogenate the sample in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. Meanwhile,

▲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 (0.01-50 U/L).
The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
Human serum	1
Rat serum	1
Rabbit serum	1
Cynomolgus monkey	1
10% Mouse liver tissue homogenate	3-5
10% Rat kidney tissue homogenate	1
10% Rat spleen tissue homogenate	5-10
10% Mouse brain 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

1. The preparation of standard curve

Dilute 500 μmol/L standard solution with reagent 1 to a serial concentration. The recommended dilution gradient is as follows: 0, 50, 100, 150, 250, 350, 400, 500 μmol/L. Reference is as follows:

Number	Standard concentrations (μmol/L)	500 μmol/L standard solution (μL)	Reagent 1 (μL)
A	0	0	200
B	50	20	180
C	100	40	160
D	150	60	140
E	250	100	100
F	350	140	60
G	400	160	40
H	500	200	0

2. 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 corresponding wells.

Control well: add 50 μL of sample into the corresponding 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.

▲Summary operation table

	Standard well	Sample well	Control well
Standards solution with different concentrations (μL)	50		
Sample (μL)		50	50
Sample working solution (μL)	50	50	
Control working solution (μL)			50
Mix fully for 5 s and incubate at 37℃ for 10 min. Measure the OD values of each well. $\Delta A = OD_{Sample} - OD_{Control}$.			

▲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: $y = ax + b$.

Serum (plasma):

Definition: the amount of enzyme in 1 L of serum (plasma) that catalyze the substrate to produce 1 μmol NAPDH at 37 ℃ for 1 min is defined as 1 unit.

$$\text{G-6-PD activity (U/L)} = (\Delta A - b) \div a \div T \times f$$

Tissue sample:

Definition: the amount of enzyme in 1 g of tissue protein that catalyze the substrate to produce 1 μmol NAPDH at 37 ℃ for 1 min is defined as 1 unit.

$$\text{G-6-PD activity (U/gprot)} = (\Delta A - b) \div a \div T \times f \div C_{pr}$$

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: $OD_{Sample} - OD_{Control}$;

f: Dilution factor of sample before test;

T: Reaction time: 10 min;

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

Appendix I Data

▲Example analysis

For mouse brain tissue, take 50 μL of mouse brain tissue homogenate, and carry the assay according to the operation table.

The results are as follows:

standard curve: y = 0.0031 x – 0.0149, the average OD value of the sample is 0.904, the average OD value of the control is 0.180, ΔA= (A_{Sample} – $A_{Control}$); the concentration of protein in sample is 7.77 gprot/L, and the calculation result is:

G-6-PD activity (U/gprot) = (0.904 - 0.180 + 0.0149) ÷ 0.0031 ÷ 10 ÷ 7.77

= 3.07 U/gprot

Appendix II References

Angelo Minucci, Bruno Giardina, and Cecilia Zuppi, et al. Critical Review: Glucose-6-phosphate Dehydrogenase Laboratory Assay: How, When, and Why[J]. IUBMB Life, 2009,61(1): 27–34.