（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K011-M

Specification：96T(40 samples)

Measuring instrument：Microplate reader（450 nm）

Detection range：5.6-500 μmol/L

Elabscience® Glucose-6-phosphate (G6P) Colorimetric Assay Kit

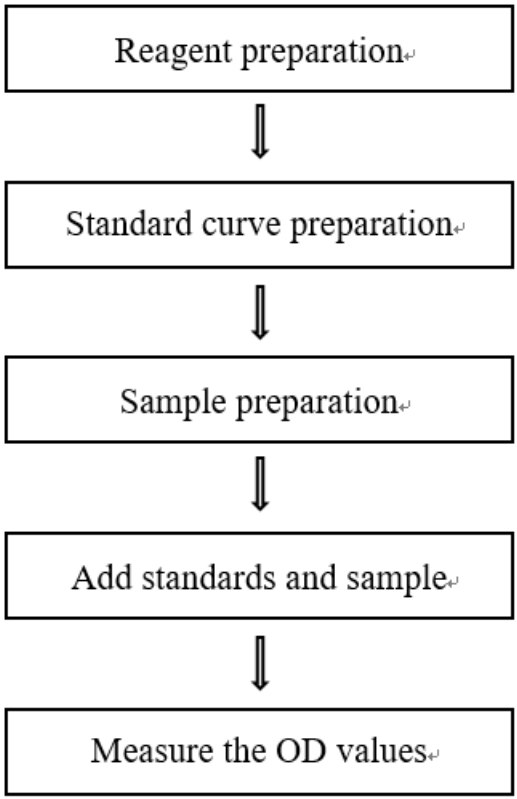
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tel: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

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

Table of contents

Assay summary



Intended use

This kit can be used to measure glucose-6-phosphate (G6P) content in serum, plasma and animal tissue samples.

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	Size (96 T)	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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

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

Reagents:

Double distilled water

Reagent preparation

① Equilibrate other reagents to room temperature before use.

② The preparation of 1 mmol/L standard solution：
Dilute 38 μL of 10 mmol/L G6P standard with 342 μL of extracting solution, mix well. The 1 mmol/L standard solution should be prepared on spot.

③ The preparation of enzyme working solution：
Dissolve one vial of enzyme agent with 1.8 mL of buffer solution, mix well. The enzyme working solution should be prepared on spot. Aliquoted storage at -20 ℃ for 7 days.

④ The preparation of control working solution：
For each well, prepare 50 uL of control working solution (mix well 25 uL of buffer solution and 25 uL of chromogenic agent). The control working solution should be prepared on spot. Store it protected from light.

⑤ The preparation of sample working solution：
For each well, prepare 50 uL of sample working solution (mix well 25 uL of chromogenic agent and 25 uL of enzyme working solution). The sample working solution should be prepared on spot. Store it protected from light.

⑥ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 1 mmol/L standard solution with extracting solution 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:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**50**	**100**	**200**	**300**	**350**	**400**	**500**
1.0 mmol/L standard (μL)	0	10	20	40	60	70	80	100
Extracting solution (μL)	200	190	180	160	140	130	120	100

Sample preparation

① Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ for a month.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL extracting solution with a dounce homogenizer at 4℃.

④ Centrifuge at 10000 × g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

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 extracting solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

Prevent the formulation of bubbles when adding the liquid to the microplate.

Operating steps

① Standard well: add 50 μL of standards with different concentrations into the standard 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 to the sample wells and standard wells.

Add 50 μL of control working solution to 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 $\Delta A = A_2 - A_1$.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

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]

ΔA_{450}: $A_2 - A_1$.

f: Dilution factor of sample before test.

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

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	10.00	185.00	375.00
%CV	2.6	2.0	1.7

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	10.00	185.00	375.00
%CV	4.2	4.5	4.2

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 95%.

	Standard 1	Standard 2	Standard 3
Expected Conc.(μmol/L)	85	265	385
Observed Conc.(μmol/L)	83.3	251.8	354.2
Recovery rate (%)	98	95	92

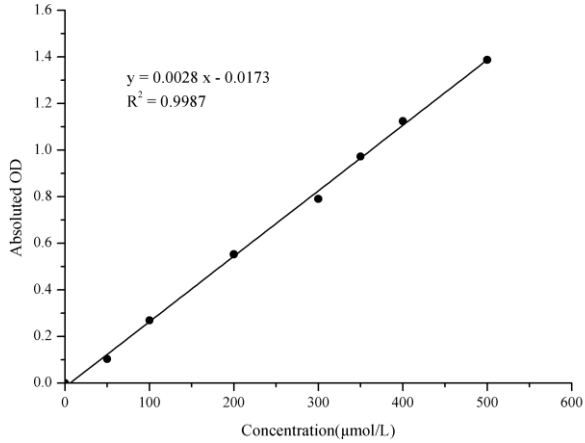
Sensitivity

The analytical sensitivity of the assay is 5.6 μmol/L G6P. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Concentration (μmol/L)	**0**	**50**	**100**	**200**	**300**	**350**	**400**	**500**
Average OD	0.055	0.158	0.324	0.608	0.845	1.027	1.179	1.442
Absoluted OD	0.000	0.103	0.269	0.553	0.791	0.973	1.124	1.388



Appendix II Example Analysis

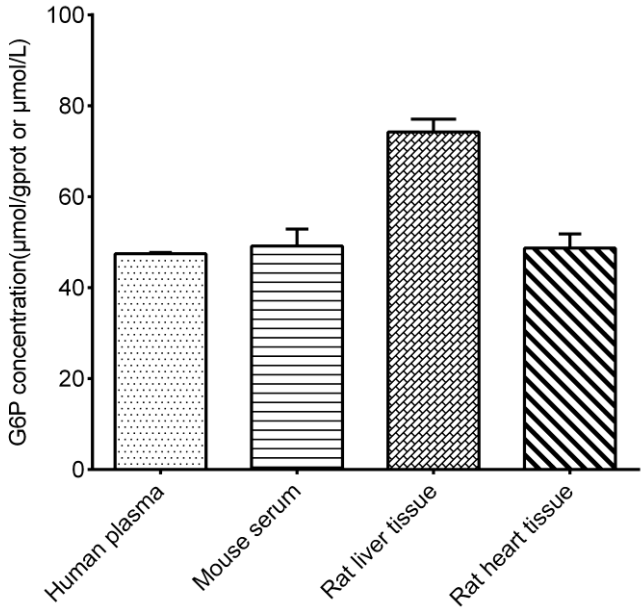
Example analysis:

For rat plasma, take 50 μL of sample into the sample wells and standard wells and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.0027 x – 0.0207, the average OD value of control is 0.370, the average OD value of the sample is 0.421, and the calculation result is:

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

Detect human plasma, mouse serum (dilute for 2 times), 10% rat liver tissue homogenate (the concentration of protein is 8.59 gprot/L dilute for 2 times), 10% rat heart tissue homogenate (the concentration of protein is 4.53 gprot/L dilute for 2 times) according to the protocol, the result is as follows:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience 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.