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

Catalog No：E-BC-K056-M

Specification：96T(40 samples)

Measuring instrument：Microplate reader（450 nm）

Detection range：0.01-50 U/L

Elabscience®Glucose-6-Phosphate Dehydrogenase (G-6-PD) Activity 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
Tell: 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.

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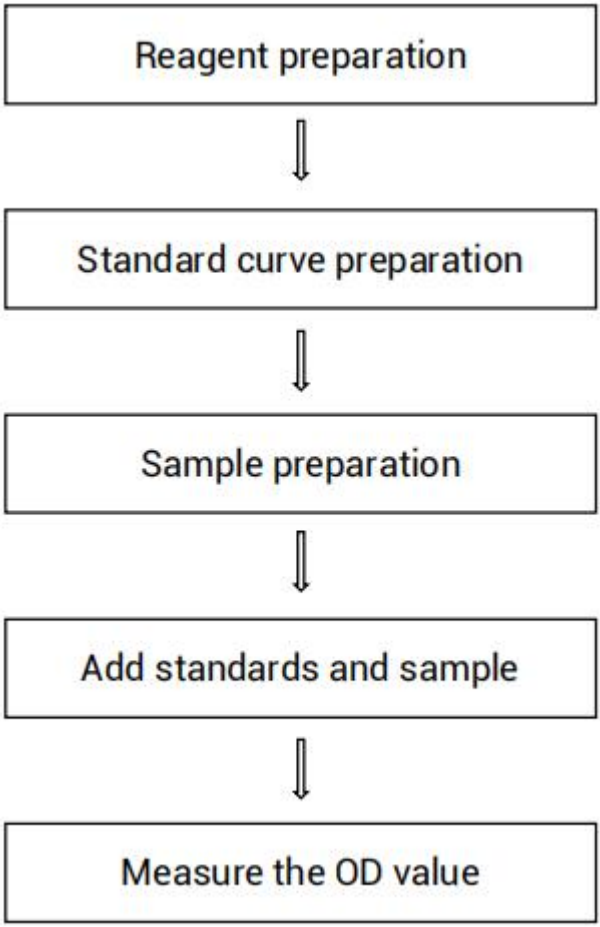
Assay summary



Intended use

This kit can be used to measure Glucose-6-Phosphate Dehydrogenase (G-6-PD) activity in serum, plasma and animal tissue samples.

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	Size (96 T)	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	
	Sample Layout Sheet	1 piece	

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), Pipettor, Water bath, Centrifuge

Reagent preparation

① Keep substrate on ice during use. Equilibrate other reagents to room temperature before use.

② The preparation of 5 mmol/L standard solution：
Dissolve one vial of standard with 720 μL of double distilled water. Mix well to dissolve. The standard solution should be prepared on spot.

③ The preparation of 500 μmol/L standard solution：
For each well，prepare 10 μL of 500 μmol/L standard solution (mix well 1 μL of 5 mmol/L standard solution and 9 μL of buffer solution). The standard solution should be prepared on spot.

④ The preparation of sample working solution：
For each well，prepare 50 μL of sample working solution (mix well 25 μL of substrate and 25 μL of chromogenic agent). The sample working solution should be prepared on spot.

⑤ The preparation of control working solution：
For each well，prepare 50 μL of control working solution (mix well 25 μL of chromogenic agent and 25 μL of buffer solution). The control working solution should be prepared on spot.

⑥ The preparation of standard curve：
Always prepare a fresh set of standards. Discard working standard dilutions after use.
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:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**50**	**100**	**150**	**250**	**350**	**400**	**500**
500 μmol/L standard (μL)	0	20	40	60	100	140	160	200
Reagent 1 (μL)	200	180	160	140	100	60	40	0

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.

Saliva: Gargle with clear water, collect the saliva 30 min later, centrifuge at 10000 g for 10 min at 4℃. Take the supernatant and preserve it on ice for detection.

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 reagent 1 with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 minutes 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 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 extracting solution. For the dilution of other sample types, please do pretest to confirm the dilution factor

The key points of the assay

① When the extracting solution 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.

Operating steps

①Standard 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.

③ Add 50 μL of control working solution into the control wells.

④ Mix fully for 5 s with microplate reader and incubate at 37℃ for 10 min.

⑤ Measure the OD values of each well at 450 nm with microplate reader.

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:

Unit 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.

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

2. Tissue sample:

Unit 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]

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 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 (U/L)	2.60	11.50	38.50
%CV	2.4	2.0	1.9

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 (U/L)	2.60	11.50	38.50
%CV	5.7	6.1	5.6

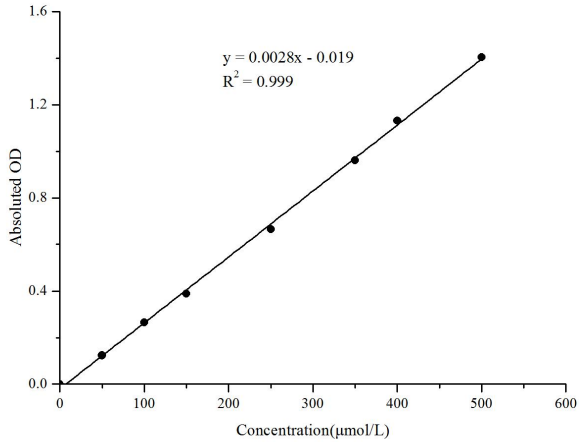
Sensitivity

The analytical sensitivity of the assay is 0.01 U/L. 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	150	250	350	400	500
Average OD	0.059	0.182	0.323	0.447	0.724	1.019	1.190	1.463
Absoluted OD	0.000	0.124	0.265	0.388	0.666	0.961	1.132	1.404



Appendix Π Example Analysis

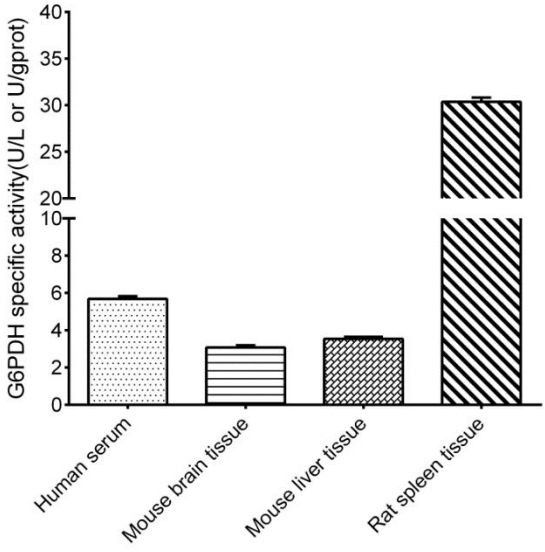
Example analysis：

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

$$\text{G-6-PD activity (U/gprot)} = (0.904 - 0.180 + 0.0149) \div 0.0031 \div 10 \div 7.77 = 3.07 \text{ U/gprot}$$

Detect human serum, 10% mouse brain tissue homogenate (the concentration of protein is 7.77 gprot/L), 10% mouse liver tissue homogenate (the concentration of protein is 11.52 gprot/L), 10% rat spleen tissue homogenate (the concentration of protein is 6.16 gprot/L) 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.