CheKine™ Micro Glucose-6-Phosphate (6PG) Content Assay Kit

Cat #: KTB1025 Size: 48 T/24 S 96 T/48 S

	Micro Glucose-6-Phosphate (6PG) Content Assay Kit		
REF	**Cat #:** KTB1025	LOT	**Lot #:** Refer to product label
	Detection range: 7.81-500 nmol/mL		**Sensitivity:** 7.81 nmol/mL
	Applicable samples: Animal and Plant Tissues, Bacteria or Cells, Plasma, Serum or other Liquid samples		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Glucose-6-phosphate (6PG), also known as Glucose 6-phosphate, is an intermediate product of glycolysis and pentose phosphate pathway, which is widely found in animals, plants and microorganisms. In the first step of glycolysis, glucose is catalyzed by hexokinase to form glucose-6-phosphate, which is then catalyzed by phosphate glucose isomerase to form fructose-6-phosphate to continue the other steps of glycolysis. However, in the pentose phosphate pathway, 6PG is its first substrate, and this process is also the main way to generate NADPH. In addition, 6PG can also be converted to glycogen or starch and stored. Glucose-6-phosphate dehydrogenase catalyzes the formation of glucose-6-phosphate and NADPH from 6PG and $NADP^+$. NADPH can make WST-8 orange-yellow under the action of 1-mPMS, and the content of 6PG can be calculated by measuring the absorbance value at 450 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	30 mL	60 mL	4℃
Reagent Ⅰ	7 mL	14 mL	4℃
Reagent Ⅱ	Powder×1 vial	Powder×1 vial	-20℃
Reagent Ⅲ	0.75 mL	1.5 mL	4℃, protected from light
Standard	1 mL	1 mL	-20℃, protected from light

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 450 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Water bath, ice machine, centrifuge, ultrasonic crusher

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- Deionized water
- Homogenizer or mortar (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.
Reagent I: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.
Reagent II: Prepare before use; 48 T was added with 2.1 mL deionized water, 96 T was added with 4.2 mL deionized water to fully dissolve; The reagents that could not be used up could be stored for 4 weeks at -20℃.
Reagent III: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃,protected from light.
Standard: Ready to use as supplied; 10 μmol/mL NADPH standard solution was stored at -20℃,protected from light.
Standard preparation: Use the 10 μmol/mL NADPH standard solution and further diluted it to the standard as shown in the following table:

Num.	Standard Volume (μL)	Deionized Water Volume（μL）	Concentration（nmol/mL）
Std.1	50 μL 10 μmol/mL Standard	950	500
Std.2	500 μL of Std.1 (500 nmol/mL)	500	250
Std.3	500 μL of Std.2 (250 nmol/mL)	500	125
Std.4	500 μL of Std.3 (125 nmol/mL)	500	62.5
Std.5	500 μL of Std.4 (62.5 nmol/mL)	500	31.25
Std.6	500 μL of Std.5 (31.25 nmol/mL)	500	15.63
Std.7	500 μL of Std.6 (15.63 nmol/mL)	500	7.81
Blank	0	500	0（Blank well）

Notes: Always prepare fresh standards per use; Diluted Standard Solution is unstable and must be used within 4 h.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month.When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.
1.Animal and Plant Tissues：Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 8,000 g for 10 min at 25℃. Use supernatant for assay, and place it on ice to be tested.
2.Bacteria or Cells：Collect 5×10^6 bacteria or cells into the centrifuge tube, wash bacteria or cells with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cells or bacteria (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 10 min at 25℃. Use supernatant for assay, and place it on ice to be tested.
3.Plasma, Serum or other Liquid samples: Direct detection.
Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 450 nm, visible spectrophotometer was returned to zero with deionized water.
2. Preparation of working reagent: according to the number of samples before use, the following proportion of working reagent is

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prepared, and it is used now.

Reagent	Test working reagent	Control working reagent
Reagent Ⅰ	100	100
Reagent Ⅱ	50	0
Deionized water	0	50
Reagent Ⅲ	10	10

3. Sample measurement. (The following operations are operated in the 96-well plate or microglass cuvette):

Reagent	Test Well（μL）	Control well（μL）	Standard Well（μL）
Sample supernatant	50	50	0
Standard	0	0	50
Test working reagent	150	0	150
Control working reagent	0	150	0

The cells were incubated at 37℃ in the dark for 30 min, and the absorbance value was measured at 450 nm, which were recorded as A_{Test}, $A_{Control}$, $A_{Standard}$ and A_{Blank}, respectively. $\Delta A_{Test}=A_{Test}-A_{control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: One control well should be set up for each assay well, and the standard curve should be measured only once or twice. Before the experiment, it is recommended to select 2-3 samples with large expected differences for pre-experiment. If ΔA_{Test} is less than 0.01, the sample size can be appropriately increased. If ΔA_{Test} is greater than 2.0, the sample can be further diluted with Extraction Buffer, the calculated result multiplied by the dilution, or the sample size used for extraction can be reduced.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Drawing of standard curve:

With the concentration of the standard solution as the x-axis and the $\Delta A_{Standard}$ as the y-axis, draw the standard curve, get the standard equation, and bring the ΔA_{Test} into the equation to get the x value (nmol/mL).

2. Calculation of glucose-6-phosphate content:

（1）Calculated by sample protein concentration:

6PG (nmol/mg prot)=$(x\times V_{sample})\div(V_{sample}\times Cpr)$**=x÷Cpr**

（2）Calculated by fresh weight of samples:

6PG (nmol/g fresh weight)=$(x\times V_{sample})\div(W\times V_{sample}\div V_{extract})$**=x÷W**

（3）Calculated by volume of liquid samples:

6PG (nmol/mL)=$(x\times V_{sample})\div V_{sample}$**=x**

（4）Calculated by bacteria or cells:

6PG (nmol/10^4)=$(x\times V_{sample})\div(500\times V_{sample}\div V_{extract})$**=x÷500**

V_{sample} :Added sample volume to the reaction system, 0.05 mL; Cpr: Sample protein concentration, mg/mL; $V_{extract}$: Added Extraction Buffer volume to the reaction system, 1 mL; W : Sample weight, g; 500: The total number of bacteria or cells, 5×10^6.

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Typical Data

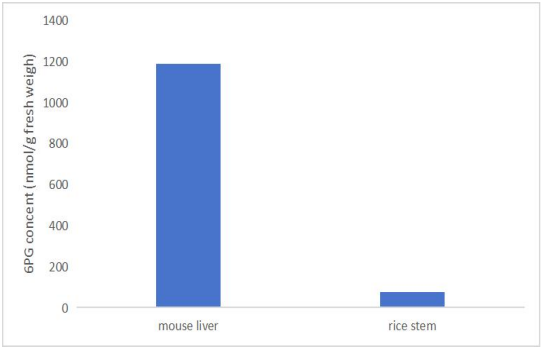

Figure 1. Determination of 6PG content in Mouse liver and Rice stem by this kit.

Recommended Products

Catalog No.	Product Name
KTB1015	CheKine™ Micro α-glucosidase(α-GC) Activity Assay Kit
KTB1121	CheKine™ Micro Pyruvate Acid (PA) Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.

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