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CheKine™ Micro Glucose-6-Phosphatase (G6P) Activity Assay Kit

Cat #: KTB1014 Size: 48 T/96 T

	Micro Glucose-6-Phosphatase (G6P) Activity Assay Kit		
REF	**Cat #:** KTB1014	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal Tissues, Plant Tissues, Serum, Plasma		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Glucose-6-phosphatase (G6Pase, EC 3.1.3.9) is widely present in animals, plants, microorganisms and cells, and is the limiting enzyme in the hydrolysis of glucose-6-phosphate by gluconeogenesis, which plays an important role in ensuring the homeostasis of blood glucose. CheKine™ Micro Glucose-6-Phosphatase (G6P) Activity Assay Kit provides a simple assay to measure G6P activity in biological samples, such as animal tissues, plant tissues, and serum (plasma) samples. G6P catalyses the production of glucose from glucose-6-phosphate, and mutase and glucose dehydrogenase further sequentially catalyse the reduction of NAD^+ to produce NADH, and the rate of NADH production was measured at 340 nm, which reflects G6P activity.

Materials Supplied and Storage Conditions

Components	Kit components	Size		Storage conditions
		48 T	96 T	
Part 1 of 2	Extraction Buffer	50 mL	100 mL	4℃
	Reagent Ⅰ	9.5 mL	19 mL	4℃
	Reagent Ⅱ	33 μL	66 μL	4℃
	Reagent Ⅲ (Not Frozen)	1 μL	2 μL	4℃, not frozen
Part 2 of 2	Reagent Ⅳ	1	1	-20℃, protected from light
	Reagent Ⅴ	1	1	-20℃, protected from light
	Reagent Ⅵ	1	1	-20℃, protected from light

Materials Required but Not Supplied

- Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 340 nm
- Incubator, freezing centrifuge

- 96-well UV plate or microquartz cuvette, precision pipettes, disposable pipette tips
- Deionized water
- Homogenizer (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℃.

Working Reagent: Prepared before use, all reagent Ⅱ, reagent Ⅲ, reagent Ⅳ, reagent Ⅴ, and reagent Ⅵ were transferred into reagent Ⅰ, fully dissolved for use. Stored aliquots at -20℃ with unused reagent, and avoid repeated freezing and thawing.

Sample Preparation

Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80℃ for one month.

1. Animal tissues samples: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Plant tissue samples: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and mash. Ultrasonic break in ice bath 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
3. Serum, Plasma samples: Tested directly.

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 ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 340 nm, Ultraviolet spectrophotometer was returned to zero with deionized water.
2. Working Reagent place at 37°C (mammal) or 25°C (other species) incubation for 5 min.
3. Add 10 μL sample, and 190 μL Working Reagent to the 96-well UV plate or microquartz cuvette, mix quickly.
4. Measure the absorbance value at 340 nm with a microplate reader, record 10 s absorbance value as A_1 and the absorbance value at 2 min 10 s as A_2, and calculate $\Delta A=A_2-A_1$.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA is less than 0.001, increase the sample quantity appropriately. If ΔA is greater than 0.3, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

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.

A. 96-well UV plates calculation formula as below

1. Calculation of G6P activity in serum (plasma)

Active unit definition: 1 nmol NADH consumed per min in 1 mL Serum (Plasma) reaction system is defined as a unit of enzyme activity.

$G6P\ (U/mL)=[\Delta A\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div V_{Sample}\div T$**$=3,215.43\times\Delta A$**

2. Calculation of G6P activity in tissues

(1) Calculated by protein concentration

Active unit definition: 1 nmol NADH consumed per min in 1mg tissue protein reaction system is defined as a unit of enzyme

activity.

G6P (U/mg prot)=[ΔA×V_{Total}÷(ε×d)×10^9]÷(Cpr×V_{Sample})÷T**=3,215.43×ΔA÷Cpr**

(2) Calculated by sample fresh weight

Active unit definition: 1 nmol NADH consumed per min in 1 g tissue reaction system is defined as a unit of enzyme activity.

G6P (U/g fresh weight)=[ΔA×V_{Total}÷(ε×d)×10^9]÷(V_{Sample}÷$V_{Total\ Sample}$×W)÷T**=3,215.43×ΔA÷W**

Where: V_{Total}: total reaction volume, 2×10^{-4} L; ε: NADH molar extinction coefficien, 6.22×10^3 L/mol/cm; d: 96-well plate diameter, 0.5 cm; 10^9: 1 mol=1×10^9 nmol; V_{Sample}: sample volume added, 0.01 mL; $V_{Total\ Sample}$: extract solution volume added, 1 mL; T: reaction time, 2 min; Cpr; sample protein concentration, mg/mL; W: sample weight, g.

B. Microquartz cuvette calculation formula

The optical diameter d: 0.5 cm in the above calculation formula can be adjusted to d: 1 cm for calculation.

Recommended Products

Catalog No.	Product Name
KTB1110	CheKine™ Micro Lactate Dehydrogenase (LDH) Assay Kit
KTB1300	CheKine™ Micro Glucose Assay Kit
KTB1100	CheKine™ Micro Lactate Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.