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CheKine™ Micro Glycogen Synthase (GCS) Activity Assay Kit

Cat #: KTB1341 Size: 48T/96 T

	Micro Glycogen Synthase (GCS) Activity Assay Kit		
REF	**Cat #:** KTB1341	LOT	**Lot #:** Refer to product label
	Applicable sample: Animal Tissues, Cells or Bacteria		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Glycogen synthase (GCS) adds the glycosyl of UDPG to the original glycogen or the non-reducing end of glycogen protein, and connects them with α-1,4 glycosidic bonds. GCS is the rate-limiting enzyme in the process of glycogen synthesis in animals, and it is also the main target enzyme of insulin, which plays an important role in the process of glucose metabolism and maintaining the relative stability of blood sugar. GCS catalyzes UDPG and glucose residues to generate glycogen and UDP, and pyruvate kinase and lactate dehydrogenase further catalyze NADH to generate NAD^+ in turn. The activity of GCS can be reflected by measuring the decline rate of NADH at 340 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	50 mL×2	4℃
Reagent Ⅰ	9 mL	18 mL	4℃
Reagent Ⅱ	1.25 mL	2.5 mL	4℃
Reagent Ⅲ	8.2 μL	16.4 μL	4℃, protected from light
Reagent Ⅳ	1	1	-20℃, protected from light
Reagent Ⅴ	1	1	-20℃, protected from light

Materials Required but Not Supplied

- Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 340 nm
- 96-well UV plate or microquartz cuvette, precision pipettes, disposable pipette tips
- Thermostatic incubator, ice maker, centrifuge, ultrasonic crusher
- Deionized water
- Homogenizer

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: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent III: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃ and protected from light.

Reagent IV: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20℃ and protected from light.

Reagent V: Prepared before use. add 0.5 mL Reagent II for 48 T and 1 mL Reagent II for 96 T to fully dissolve. The remaining reagent can also be stored at -20℃ and protected from light after aliquoting to avoid repeated freezing and thawing.

Working Reagent: Transfer all Reagent III and Reagent IV to Reagent I before use, and fully dissolve them. Store the inexhaustible reagents at -20℃ and protected from light after aliquoting to avoid repeated freezing and thawing.

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 tissues: Weigh about 0.1 g of sample, add 1 mL of pre cooled Extraction Buffer, homogenize in ice bath, centrifuge at 8,000 g, 4℃ for 10 min, take the supernatant, and put it on ice for testing.

2. Cells or Bacteria: Collect 5 million cells or bacteria into a centrifuge tube, wash the cells with cold PBS, discard the supernatant after centrifugation, add 1 mL of pre cooled Extraction Buffer, break the cells or Bacteria with ice bath ultrasonic for 5 min (power 20% or 200 W, ultrasonic for 3 s, interval 7 s, repeat 30 times), then centrifuge at 8,000 g, 4℃ for 10 min, take the supernatant, and put it on ice for testing.

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. According to the dosage, preheat the prepared Working Reagent and Reagent V at 37℃ for 5 min.

3. Add 10 μL of sample supernatant, 10 μL of Reagent V and 180 μL Working Reagent into a 96-well UV plate or a microquartz cuvette, immediately mix them evenly, record the initial absorbance A1 at 340 nm and the absorbance A2 after 1 min, and calculate ΔA=A1-A2.

Note: Before the experiment, it is recommended to select 2-3 samples with large expected differences for pre experiment. If ΔA is greater than 0.1, the sample can be further diluted with Extraction Buffer, and the calculated result is multiplied by the dilution factor, or the sample size 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.

A. 96-well UV plates calculation formula as below

1. Calculated by protein concentration

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

GCS (U/mg prot)=$[\Delta A\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(V_{Sample}\times Cpr)\div T$**=6,340×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.

GCS (U/g fresh weight)=$[\Delta A\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(V_{Sample}\div V_{Total\ Sample}\times W)\div T$**=6,340×ΔA÷W**

3. Calculated by bacteria or cell number

Active unit definition: 1 nmol NADH consumed per min in 10^4 bacteria or cells reaction system is defined as a unit of enzyme activity.

GCS ($U/10^4$)=[$\Delta A \times V_{Total} \div (\varepsilon \times d) \times 10^9$]÷($V_{Sample} \div V_{Total\ Sample} \times 500$)÷T=6340×ΔA÷500**=12.68×ΔA**

V_{Total}: total reaction volume, 2×10^{-4} L; ε: NADH molar extinction coefficien, 6.22×10^3 L/mol/cm; d: 96-well UV plate diameter, 0.5 cm; 10^9: 1 mol=1×10^9 nmol; V_{Sample}: sample volume added, 0.01 mL; $V_{Total\ Sample}$: Extraction Buffer volume added, 1 mL; T: reaction time, 1 min; Cpr; sample protein concentration, mg/mL; W: sample weight, g; 500: Total number of bacteria or cells, 5×10^6.

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.

Typical Data

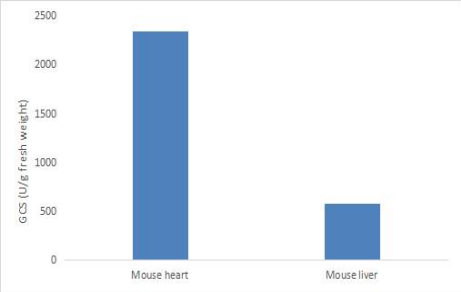

Figure 1. Determination GCS activity in mouse heart and mouse liver by this assay kit

Recommended Products

Catalog No.	Product Name
KTB1111	CheKine™ Micro D-lactate Dehydrogenase (D-LDH) Activity Assay Kit
KTB1121	CheKine™ Pyruvate Acid (PA) Colorimetric Assay Kit

Disclaimer

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