CheKine™ Micro Glucose Dehydrogenase (GCDH) Activity Assay Kit

Cat #: KTB1026 Size: 48 T/48 S 96 T/96 S

	Micro Glucose Dehydrogenase (GCDH) Activity Assay Kit		
REF	**Cat #:** KTB1026	LOT	**Lot #:** Refer to product label
	Applicable samples: Higher Animal Tissues, Serum, Plasma or other Liquids		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

GCDH (EC 1.1.1.47) catalyzes D-glucose and NAD(P) to form D-Gluconic acid and NAD(P)H, which are mainly found in the liver of many microorganisms and higher animals. The use of GCDH in the production of oligofructose not only removes glucose from oligofructose and increases its content, but also generates gluconic acid that combines with calcium ions to form calcium gluconate, which is an ideal calcium supplement. Therefore, GCDH has become an ideal enzyme for preparing high content oligofructose. GCDH catalyzes the formation of d-Gluconic acid and NADH from D-glucose and NAD. The activity of glucose dehydrogenase can be reflected by the change of absorbance value of NADH at 340 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	60 mL	60 mL×2	4℃
Reagent Ⅰ	12 mL	24 mL	4℃
Reagent Ⅱ	Powder×1 vial	Powder×1 vial	-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 ultraviolet spectrophotometer capable of measuring absorbance at 340 nm
- 96-well UV plate or microquartz cuvette, precision pipettes, disposable pipette tips
- Water bath, ice maker, centrifuge
- 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 Ⅱ: Prepare before use, 48 T add 11.4 mL Reagent Ⅰ, 96 T add 22.8 mL Reagent Ⅰ to dissolve it for use. The unused Working Reagent Ⅱ can be stored at 4℃, protected from light for one week.

Sample Preparation

Note: It is recommended to use fresh samples. If the experiment is not conducted immediately, the samples can be stored at -80℃ for 1 month. The temperature and time of thawing should be controlled during the determination. When thawing at room temperature, the sample should be thawed within 4 h.

1. Tissues: Weigh 0.1 g tissue , add 1 mL Extraction Buffer, homogenize on ice. Centrifuge at 8,000 g for 10 min at 4℃. Take the supernatant and place it on the ice for testing.
2. Serum, Plasma and other Liquid Samples: Direct detection. If the solution has turbidity, centrifuge and take the supernatant for measurement.

Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine catalog number: 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. Preheat the prepared Reagent Ⅱ at 37℃ for 5 min.
3. Sample measurement. (The following operations are operated in the 96-well UV plate or microquartz cuvette)

Regent	Test well (μL)
Sample	10
Working Reagent Ⅱ	190
Mix well, record the absorbance values of 10 s and 1 min 10 s at 340 nm, mark as A_1 and 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 the ΔA is less than 0.01, the sample size can be appropriately increased or the reaction time can be appropriately extended to 5 min or 10 min for detection. If the ΔA is greater than 1.5, the sample can be appropriately diluted with Extraction Buffer or reduce the sample quality used for extraction, the calculated result multiplied by the dilution factor.

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

(1) Calculated by protein concentration:

Unit definition: One enzyme activity unit defines as 1 nmol NADH produced by 1 mg tissue proteins per min.

GCDH (U/mg prot)=$[\Delta A\times V_{Total}\div(\varepsilon\times d)\times10^9]\div(V_{Sample}\times Cpr)\div T$**=6,430×ΔA÷Cpr**

(2) Calculation according to the weight of the sample:

Unit definition: One enzyme activity unit defines as 1 nmol NADH produced by 1 g tissue per min

GCDH (U/g fresh weight)=$[\Delta A\times V_{Total}\div(\varepsilon\times d)\times10^9]\div(V_{Sample}\times W\div V_{Sample\ Total})\div T$**=6,340×ΔA÷W**

(3) Calculation according to the volume of liquid:

Unit definition: One enzyme activity unit defines as 1 nmol NADH produced by 1 mL liquid per min

GCDH (U/mL)=$[\Delta A\times V_{Total}\div(\varepsilon\times d)\times10^9]\div V_{Sample}\div T$**=6,340×ΔA**

Where: ε: NADH molar extinction coefficient, 6.22×10^3 L/mol/cm; d: 96-well UV plate diameter, 0.5 cm; V_{Total}: the total volume of

the reaction system, 0.2 mL=2×10^{-4} L; V_{Sample}: the volume of the sample in the reaction system, 0.01 mL; $V_{Sample\ Total}$: The volume of Extraction Buffer added, 1 mL; Cpr: protein concentration (mg/mL); W: sample weight, g; T: reaction time, 1 min.

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.

Precautions

1. The extracted sample supernatant is placed on the ice to be tested, and it is recommended to complete the test on the same day after the sample extraction is completed.

Typical Data

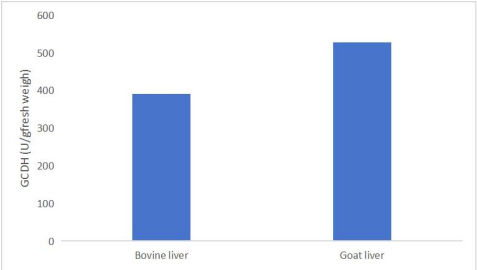

Figure 1. GCDH activity in Bovine liver and Goat liver was detected with this kit

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.