CheKine™ Micro Glucokinase (GCK) Activity Assay Kit

Cat #: KTB1131 Size: 48 T/48 S 96 T/96 S

	Micro Glucokinase (GCK) Activity Assay Kit		
REF	**Cat #:** KTB1131	LOT	**Lot #:** Refer to product label
≠	**Detection range:** 15.625-1000 μM		**Sensitivity:** 15.625 μM
	Applicable sample: Animal and Plant Tissues, Cells, Bacteria, Serum, Plasma and other liquid samples		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Glucokinase (GCK) is a key enzyme in the glycolytic pathway, participating in the first step of glycolysis catalyzing the phosphorylation of glucose, promoting insulin secretion and glucose metabolism, and effectively controlling blood glucose balance in the body. GCK is also known as hexokin IV, mainly expressed in hepatocytes and islet beta cells, and has a higher affinity for glucose than other hexokinases. Therefore, under high sugar concentration, the measured is the total hexokinase activity in the sample, and under low sugar concentration, the measured value is the activity of hexokinase with low affinity for glucose. The GCK in the sample can be obtained by subtracting the activity of hexokinase from the total hexokinase. The GCK catalyzes the reaction of substrate to produce NADPH, and the generated NADPH reduces WST-8 to form orange-yellow formazan, which has a maximum absorption peak at around 450 nm. The increase rate of light absorption at 450 nm is detected to calculate the activity of GCK in the sample.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	60 mL	60 mL×2	4℃
Reagent One	30 mL	60 mL	4℃
Reagent Two	powder	powder	-20℃, protected from light
Reagent Three	powder	powder	4℃
Reagent Four	powder	powder×2	-20℃, protected from light
Reagent Five	0.75 mL	1.5 mL	-20℃, protected from light
Reagent Six	150 μL	300 μL	-20℃, protected from light
Standard	powder	powder	-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
- Incubator, ice maker, low-temperature centrifuge
- PBS, Deionized water

Reagent Preparation

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

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

Working Reagent Two: Prepared before use. Add 13 mL Reagent one to the 48 T bottle to fully dissolve, add 26 mL Reagent one to the 96 T bottle to fully dissolve. Stored at -20℃, protected from light for one month, avoid repeated freezing and thawing.

Working Reagent Three: Prepared before use. Add 6.5 mL Reagent one to the 48 T bottle to fully dissolve, add 13 mL Reagent one to the 96 T bottle to fully dissolve. Stored at 4℃.

Working Reagent Four: Prepared before use. Add 0.5 mL Reagent one to each bottle of Reagent Four to fully dissolve. Stored at -20℃, protected from light for one month, avoid repeated freezing and thawing.

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

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

Standard: Prepared before use. Dissolve completely with 1 mL deionized water, the concentration is 2000 μM. Stored at -20℃, protected from light for one month.

Setting of Standard curves: Before use, dilute 2000 μM standard with deionized water to 1000 μM, 500 μM, 250 μM, 125 μM, 62.5 μM, 15.625 μM standard solution as shown in the table below.

Num.	Volume of Standard (μL)	Volume of deionized water (μL)	Concentration (μM)
Std.1	100 μL 2000 μM	100	1000
Std.2	100 μL 1000 μM	100	500
Std.3	100 μL 500 μM	100	250
Std.4	100 μL 250 μM	100	125
Std.5	100 μL 125 μM	100	62.5
Std.6	100 μL 62.5 μM	100	31.25
Std.7	100 μL 31.25 μM	100	15.625
Blank	0	100	0

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month.

1. Animal and Plant Tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 8000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

2. Cells or Bacteria: Collect 5×10^6 cells or bacteria into the centrifuge tube, wash cells or bacteria with cold PBS, centrifuge and discard the supernatant; add 1 mL Extraction Buffer to ultrasonically disrupt the cells or bacteria 5 min on ice (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

3. Serum (Plasma): Test 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 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 the Test Working Solution: 190 μL of working solution is required per well. To avoid loss, prepare 193.5 μL per single-well system: 92 μL of Working Reagent Two, 92 μL of Working Reagent Three, 3.5 μL of Working Reagent Four, 5 μL of Reagent Five, and 1 μL of Reagent Six, mix well. The test working solution should be prepared fresh and used immediately.
3. Preparation of the Control Working Solution: 190 μL of working solution is required per well. To avoid loss, prepare 193.5 μL per single-well system: 91 μL of Reagent One, 92 μL of Working Reagent Two, 1 μL of Working Reagent Three, 3.5 μL of Working Reagent Four, 5 μL of Reagent Five, and 1 μL of Reagent Six, mix well. The control working solution should be prepared fresh and used immediately.
4. Operation table (The following operations are operated in the 96-well plate or microglass cuvette):

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)	Control Well (μL)
Deionized Water	10	0	0	0
Different Concentration of Stds.	0	10	0	0
Sample	0	0	10	10
Test Working Solution	190	190	190	0
Control Working Solution	0	0	0	190

5. The reaction system was mixed well and incubated at room temperature for 5 min.
6. Absorbance detection: Read the absorbance at 450 nm, marked as A_{blank}, $A_{standard}$, A_{Test} and $A_{Control}$ respectively.

Note: Blank Well and Standard Well only need to be measured 1-2 times. Before the experiment, it is recommended to select 2-3 samples with large expected differences for pre-experiment. If ΔA_{Test} is greater than 1.0, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor. If ΔA_{Test} is less than 0.01, improve the sample size 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.

1. Calculate $\Delta A_{standard} = A_{standard} - A_{blank}$, $\Delta A_{test} = A_{test} - A_{control}$.
2. Drawing of standard curve

With the concentration of the standard solution as the y-axis and the $\Delta A_{Standard}$ as the x-axis, draw the standard curve. The y (μM) was obtained by substituting the ΔA_{test} into the equation.

3. Calculation of GCK activity in the samples

(1) Calculated by fresh weight of samples:

Active unit definition: One unit of enzyme activity is defined as the amount of enzyme which catalyzes the production of 1 nmol of NADPH per minute per gram of tissue..

GCK(U/g fresh weight)=$y \times V_{standard} \div V_{Sample} \times V_{Extraction} \div W \div T \times n$=**$0.2 \times y \div W \times n$**

(2) Calculated by bacteria or cell number

Active unit definition: One unit of enzyme activity is defined as the amount of enzyme which catalyzes the production of 1 nmol of NADPH per minute per 10^4 bacteria or cells.

GCK(U/10^4)=$y \times V_{standard} \div V_{Sample} \times V_{Extraction} \div 500 \div T \times n$=**$0.0004 \times y \times n$**

(3) Calculation by liquid volume

Active unit definition: One unit of enzyme activity is defined as the amount of enzyme which catalyzes the production of 1 nmol of NADPH per minute per milliliter of liquid.

GCK(U/mL)= $y \times V_{standard} \div V_{Sample} \div T \times n$=**0.2×y×n**

(4) Calculated by protein concentration

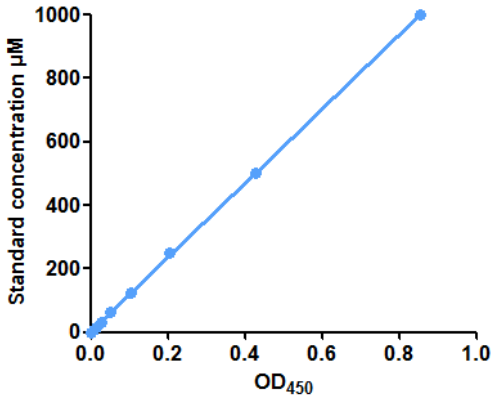
Active unit definition: One unit of enzyme activity is defined as the amount of enzyme which catalyzes the production of 1 nmol of NADPH per minute per mg protein.

GCK(U/mg prot)=$y \times V_{standard} \div V_{Sample} \div Cpr \div T \times n$=**0.2×y÷Cpr×n**

nmol： 1 μM=1 nmol/mL; $V_{standard:}$ Standard volume added to the reaction system, 10 μL; $V_{Sample:}$ Sample volume added to the reaction system, 10 μL; $V_{Extraction:}$ Extraction volume added to the reaction system, 1 mL; W: Sample weight, g; T： Reaction time，5 min; n: Sample dilution factor; Cpr: Sample protein concentration, mg/mL; 500: Total number of bacteria or cells, 5×10^6.

Typical Data

Typical standard curve: y=1169.6x+2.634， R^2=0.9999



Example-1: 0.1 g of mouse heart tissue, operate according to the measurement steps, and detect with a 96-well plate. Measured: $\Delta A_{test} = A_{test} - A_{control}$.= 1.0370 - 0.9033 = 0.1337. Substitute the ΔA_{Test} into the equation to obtain the y=159.01, Calculated by fresh weight of samples: GCK(U/g fresh weight)=$y \times V_{standard} \div V_{Sample} \times V_{Extraction} \div W \div T \times n$=**0.2×y÷W×n=318.02 U/g.**

Recommended Products

Catalog No.	Product Name
KTB1110	CheKine™ Micro Lactate Dehydrogenase (LDH) Activity Assay Kit
KTB1120	CheKine™ Micro Pyruvate Kinase (PK) Assay Kit
KTB1122	CheKine™ Micro Phosphoenolpyruvate Carboxylase (PEPC) Activity Assay Kit
KTB1123	CheKine™ Micro Hexokinase (HK) Activity Assay Kit
KTB1300	CheKine™ Micro Glucose Assay Kit
KTB1310	CheKine™ Micro Glucose oxidase (GOD) Activity 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.