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CheKine™ Micro Glycogen Phosphorylase a (GPa) Activity Assay Kit

Cat #: KTB1342 Size: 48 T/96 T

	Micro Glycogen Phosphorylase a (GPa) Activity Assay Kit		
REF	**Cat #:** KTB1342	LOT	**Lot #:** Refer to product label
	Applicable sample: Animal tissues, Cells or Bacteria, Plasma, Serum or other Liquid samples		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Glycogen phosphorylase is divided into active glycogen phosphorylase a (Glycogen phosphorylase a, GPa) and inactive glycogen phosphorylase b (Glycogen phosphorylase b, GPb) two forms. The decomposition of glycogen is mainly carried out under the catalysis of glycogen phosphorylase a. CheKine™ Micro Glycogen Phosphorylase a (GPa) Activity Assay Kit can detect animal tissues, cells or bacteria, plasma, serum or other liquid samples. In this kit, when no activator is added, GPa catalyzes the production of glucose residues from glycogen and inorganic phosphorus to glycogen and glucose 1-phosphate. Under the action of phosphoglucose mutase and 6-phosphate glucose dehydrogenase, it further catalyzes the reduction of NADP to NADPH. Measuring the rate of increase of NADPH at 340 nm can reflect the activity of GPa.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Reagent Ⅰ	8 mL	16 mL	4℃, protected from light
Reagent Ⅱ	1	1	-20℃, protected from light
Reagent Ⅲ	1	1	-20℃, protected from light
Reagent Ⅳ	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 microplate or microquartz cuvette, precision pipettes, disposable pipette tips
- Water bath, cryogenic centrifuge,1.5 mL EP tube
- Deionized water
- Mortar or homogenizer (for tissue samples)

Reagent Preparation

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

Working Reagent: Prepared before use. Transfer Reagent II to Reagent I and fully dissolve. The remaining reagent can also be stored at -20℃ and protected from light for 1 month after aliquoting to avoid repeated freezing and thawing.

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

Reagent IV: Prepared before use. Add 0.5 mL deionized water for 48 T and 1 mL deionized water for 96 T to fully dissolve. The remaining reagent can also be stored at -20℃ and protected from light for 1 month 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 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. Cells or Bacteria: Collect 5×10^6 cells or bacteria into the centrifuge tube, wash cells or bacteria with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cells or bacteria 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 or other Liquid samples: 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 ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 340 nm, ultraviolet spectrophotometer was returned to zero with deionized water.
2. Operation table (The following operations are operated in the 96-well UV microplate or microquartz cuvette):

Reagent	Test Well (μL)
Sample	10
Reagent III	10
Reagent IV	10
Deionized Water	10
Working Reagent	160

3. Mix thoroughly, measure the absorbance value A_1 at 10 s at 340 nm, and the absorbance value A_2 at 610 s at 37℃ for 10 min. Finally 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 0.05, increase the sample quantity appropriately. If ΔA is greater than 0.6, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately. If ΔA is negative, the sample does not contain GPa or is degraded.

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.

Calculation of GPa activity:

A. 96-well UV plates calculation formula as below

(1) Calculated by protein concentration

Active unit definition: The production of 1 nmol of NAPDH per milligram of protein per min was defined as one unit of enzyme activity.

GPa (U/mg prot)=[ΔA×V_{Total}÷(ε×d)×10^9]÷(V_{Sample}×Cpr)÷T=**643×ΔA÷Cpr**

(2) Calculated by fresh weight of samples

Active unit definition: The production of 1 nmol of NAPDH per gram tissue per min was defined as one unit of enzyme activity.

GPa (U/g fresh weight)=[ΔA×V_{Total}÷(ε×d)×10^9]÷(W×V_{Sample}÷$V_{Total\ sample}$)÷T=**643×ΔA÷W**

(3) Calculated by bacteria or cell number

Active unit definition: The production of 1 nmol of NAPDH per 10^4 bacteria or cell min was defined as one unit of enzyme activity.

GPa (U/10^4) = [ΔA×V_{Total}÷(ε×d)×10^9]÷(n×V_{Sample}÷$V_{Total\ sample}$)÷T=**643×ΔA÷n**

(4) Calculated by volume of samples

Active unit definition: The production of 1 nmol of NAPDH per mL liquid per min was defined as one unit of enzyme activity.

GPa (U/mL) = [ΔA×V_{Total}÷(ε×d)×10^9]÷(V_{Sample}÷$V_{Total\ sample}$)÷T=**643×ΔA**

V_{Total}: total reaction volume, 2×10^{-4} L; ε: NADPH molar extinction coefficient, 6.22×10^3 L/mol /cm; d: the light path of the 96-well UV plate, 0.5 cm; V_{Sample}: sample volume added, 0.01 mL; $V_{Total\ sample}$: Extraction Buffer volume added, 1 mL; T: reaction time, 10 min; Cpr: sample protein concentration, mg/mL; W: weight of sample, g; n: Total number of bacteria or cells, calculated in units of ten thousand.

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

The following data are for reference only. And the experimenters need to test the samples according to their own experiments.

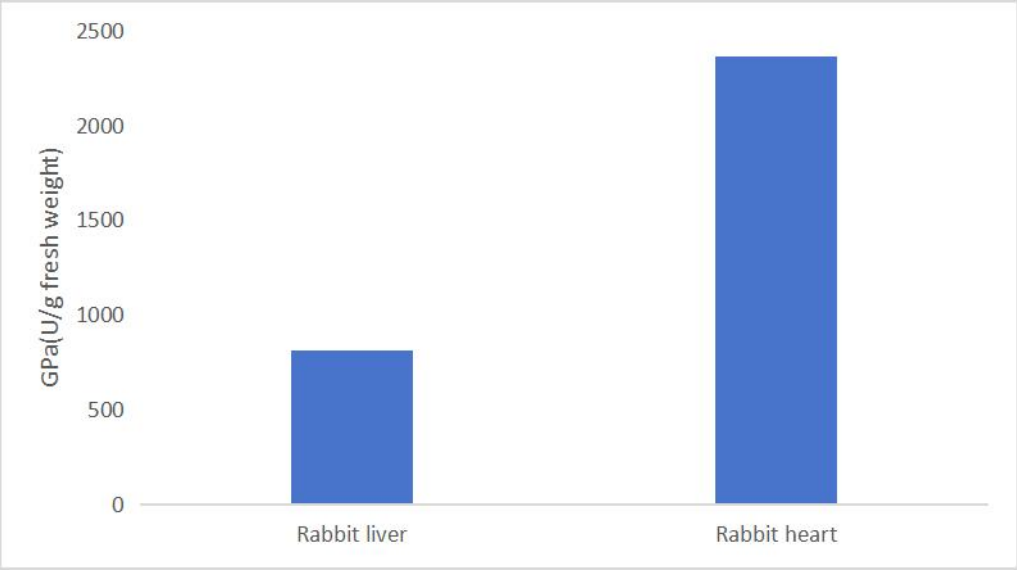

Figure 1. Determination GPa activity in rabbit liver and heart by this assay kit.

Recommended Products

Catalog No.	Product Name
KTB3030	CheKine™ Micro Alcohol Dehydrogenase (ADH) Activity Assay Kit
KTB1560	CheKine™ Micro Alcohol Acyltransferase (AAT) Activity Assay Kit
KTB1270	CheKine™ Micro Pyruvate Dehydrogenase (PDH) Activity Assay Kit

Disclaimer

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