CheKine™ Micro Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) Activity Assay Kit

Cat #: KTB1001 Size: 48 T/96 T

	Micro Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) Activity Assay Kit		
REF	**Cat #:** KTB1001	LOT	**Lot #:** Refer to product label
	Applicable sample: Animal and Plant Tissues, Cells or Bacteria, Culture Solution or other Liquid samples		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) (EC1.2.1.12) Glyceraldehyde-3-phosphoric acid catalyzed oxidation produce 1,3-diphosphate glyceric acid, is the key enzyme of glycolytic pathway, It is closely related to gluconeogenesis pathway, maintenance of blood glucose concentration in the body and the occurrence of diabetes, and plays an important role in the disorders of sugar, lipid and protein metabolism. CheKine™ Micro Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) Activity Assay Kit can detect animal and plant tissues, cells or bacteria, culture solution or other liquid samples. In this kit, 3-phosphoglycerate kinase catalyzes the formation of 1,3-diphosphoglycerate from triphosphoglycerate and ATP. GAPDH reversely catalyzes 1,3-diphosphoglyceric acid and NADH to produce glyceraldehyde 3-phosphate, inorganic phosphorus and NAD^+. The decrease of NADH measured at 340 nm can reflect the level of GAPDH activity.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Reagent Ⅰ	10 mL	20 mL	4℃, protected from light
Reagent Ⅱ	1	1	-20℃, protected from light
Reagent Ⅲ	100 μL	200 μL	4℃, 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 micro quartz cuvette, precision pipettes, disposable pipette tips, 1.5 mL EP tube
- Water bath, cryogenic centrifuge
- Deionized water
- Mortar or homogenizer (for tissue samples)

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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℃, protected from light.

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

Working Reagent: Prepared before use. Transfer Reagent Ⅱ to Reagent Ⅰ bottle 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 Ⅲ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

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. Tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize or mortar on ice. Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay.
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.
3. Culture solution or other Liquid samples: Test directly.

Note: It will be better to quantify the total protein with Protein Quantification Kit (Bradford Assay), Cat #: KTD3002, if it is calculated by protein concentration.

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℃ incubation for 10 min.
3. Operation table (The following operations are operated in the 96-well UV microplate or micro quartz cuvette):

Reagent	Test Well (μL)	Blank Well (μL)
Sample	4	0
Deionized water	0	4
Working Reagent	194	194
Reagent Ⅲ	2	2

4. Mix quickly, measure the absorbance value A_1 at 10 s at 340 nm, and the absorbance value A_2 at 310 s at 37℃ for 5 min. The Test Well is marked as A_{Test}, and the Blank Well is marked as A_{Blank}. Finally calculate $\Delta A=(A_{2Test}-A_{1Test})-(A_{2Blank}-A_{1Blank})$.

Note: The Blank Well only need to be done 1-2 times. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA is less than 0.01, 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 GAPDH 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.

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Calculation of GAPDH activity:

A. 96-well UV plates calculation formula as below

(1) Calculated by protein concentration

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

GAPDH (U/mg prot)=$[\Delta A\times V_{Total}\div(\varepsilon\times d)\times10^9]\div(V_{Sample}\times Cpr)\div T$**=3,215.43×ΔA÷Cpr**

(2) Calculated by fresh weight of samples

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

GAPDH (U/g fresh weight)=$[\Delta A\times V_{Total}\div(\varepsilon\times d)\times10^9]\div(W\times V_{Sample}\div V_{Total\ sample})\div T$**=3,215.43×ΔA÷W**

(3) Calculated by bacteria or cells

Active unit definition: The consumption of 1 nmol of NADH per 10^4 bacteria or cells per min was defined as one unit of enzyme activity.

GAPDH (U/10^4)=$[\Delta A\times V_{Total}\div(\varepsilon\times d)\times10^9]\div(W\times V_{Sample}\div V_{Total\ sample})\div T$**=3,215.43×ΔA÷W**

(4) Calculated by volume of liquid samples

Active unit definition: The consumption of 1 nmol of NADH per mL liquid was defined as one unit of enzyme activity.

GAPDH (U/mL)=$[\Delta A\times V_{Total}\div(\varepsilon\times d)\times10^9]\div(V_{Sample}\div V_{Total\ sample})\div T$**=3,215.43×ΔA**

V_{Total}: total reaction volume, 0.2 mL=0.0002 L; ε: NADPH molar extinction coefficient, 6.22×10^3 L/mol /cm; d: the light path of the 96-well plate, 0.5 cm; 10^9: 1 mol=1×10^9 nmol; V_{Sample}: sample volume added, 0.004 mL; $V_{Total\ sample}$: added Extraction Buffer volume , 1 mL; T: reaction time, 5 min; Cpr: sample protein concentration, mg/mL; W: weight of sample;g n: 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.

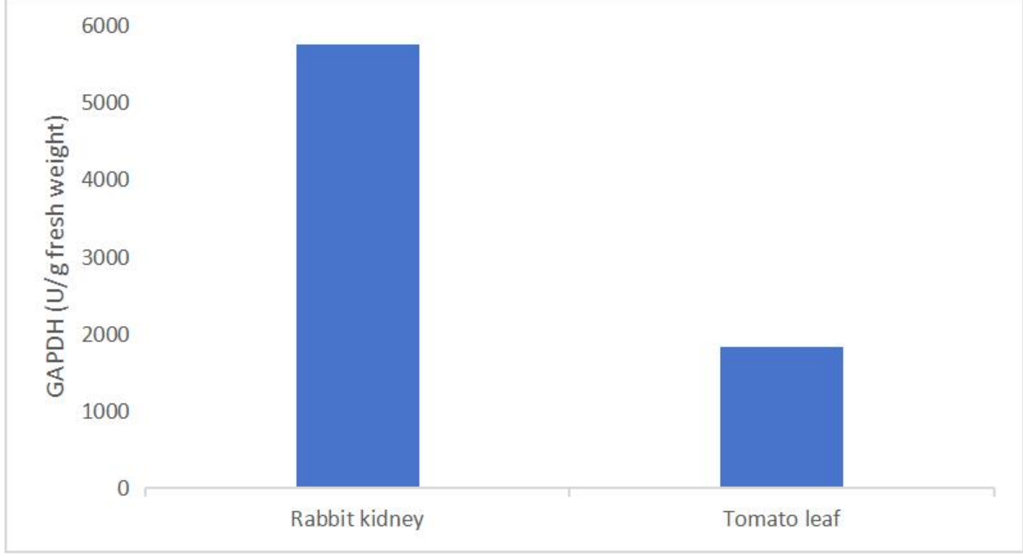

Figure 1. Determination GAPDH activity in rabbit kidney and tomato leaf by this assay kit.

Recommended Products

Catalog No.	Product Name
KTB3030	CheKine™ Micro Alcohol Dehydrogenase (ADH) Activity Assay Kit

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KTB1560	CheKine™ Micro Alcohol Acyltransferase (AAT) Activity Assay Kit
KTB1270	CheKine™ Micro Pyruvate Dehydrogenase (PDH) Activity Assay Kit

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