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CheKine™ Micro 6-Phosphogluconate Dehydrogenase Activity Assay Kit

Cat #: KTB1013 Size: 48 T/96 T

	Micro 6-Phosphogluconate Dehydrogenase Activity Assay Kit		
REF	**Cat #:** KTB1013	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal and Plant Tissues, Cells, Bacteria, Serum, Plasma		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

In the pentose phosphate pathway, glucose 6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6PGDH) catalyze the synthesis of NADPH in turn, which are closely related to energy balance, growth rate and cell viability. In addition, 6PGDH plays an important role in stress physiology. CheKine™ Micro 6-Phosphogluconate Dehydrogenase Activity Assay Kit provides a simple, convenient and rapid 6PGDH activity detection method, which is suitable for the detection of animal tissues, plant tissues, cells, bacteria, serum, plasma and other samples. The principle is that 6PGDH catalyzes 6-phosphogluconic acid and $NADP^+$ to generate NADPH, NADPH has a characteristic absorption peak at 340 nm, but $NADP^+$ does not; 6PGDH activity is calculated by measuring the increase rate of absorbance at 340 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Reagent Ⅰ	9.5 mL	19 mL	4℃
Reagent Ⅱ	1	1	-20℃, protected from light
Reagent Ⅲ	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
- Ice maker, refrigerated centrifuge, incubator
- 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 Solution: Prepare before use. Transfer all Reagent II and Reagent III to Reagent I and mix well; The remaining reagents should be stored at -20℃ and protected from light after aliquoting to avoid repeated freezing and thawing.

Sample Preparation

Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80℃ for one month. All samples and reagents should be on ice to avoid denaturation and deactivation.

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. Plant tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and mash. Ultrasonic break in ice bath 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. Cells or bacteria: Collect 2×10^7 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.

4. Plasma and serum: Direct detection.

Note: It will be better to quantify the total protein with Protein Quantification Kit (BCA Assay), Cat #: KTD3001, if the content 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. Incubate Working Solution for 10 min at 37℃ (mammal) or 25℃ (other species).

3. Sample measurement (The following operations are operated in the 96-well UV plate or microquartz cuvette).

Reagent	Blank Well（μL）	Test Well（μL）
Sample	0	10
Deionized Water	10	0
Working Solution	190	190

4. After mixing quickly, record the absorbance values of 10 s and 3 min 10 s at 340 nm with a microplate reader, mark as A_1 and A_2, and calculate $\Delta A_{Test}=(A_{Test2}-A_{Test1})-(A_{Blank2}-A_{Blank1})$.

Note: Blank well only needs to measure 1 time. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is greater than 0.5, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor. It is not suggested to test too many samples at the same time, because enzyme activity is calculated by the variation of absorbance value per unit time.

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. Calculation of 6PGDH activity in serum (plasma)

Unit definition: one enzyme activity unit defines as 1 nmol NADPH produced by each milliliter of serum (plasma) per min.

6PGDH (U/mL)= $[\Delta A_{Test}\times V_{total}\div(\varepsilon\times d)\times10^9]\div V_{sample}\div T$**$=2,144\times\Delta A_{Test}$**

2. Calculation of 6PGDH activity in tissue of the sample

(1) Calculation according to the protein concentration of the sample

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

6PGDH (U/mg prot)=[ΔA_{Test}×V_{total}÷(ε×d)×10^9]÷(V_{sample}×Cpr)÷T**=2,144×ΔA_{Test}÷Cpr**

(2) Calculation according to the weight of the sample

Unit definition: one enzyme activity unit defines as 1 nmol NADPH produced by 1 g tissue per min.

6PGDH (U/g fresh weight)=[ΔA_{Test}×V_{total}÷(ε×d)×10^9]÷(W÷$V_{extraction}$×V_{sample})÷T**=2,144×ΔA_{Test}÷W**

3. Calculation of 6PGDH activity in bacteria or cells

Unit definition: one enzyme activity unit defines as 1 nmol NADPH produced by 10^4 cells or bacteria per min.

6PGDH (U/10^4)=[ΔA_{Test}×V_{total}÷(ε×d)×10^9]÷(V_{sample}÷$V_{extraction}$×2,000)÷T**=1.07×ΔA_{Test}**

Where: V_{total}: the total volume of the reaction system, 0.2 mL=2×10^{-4} L, $V_{Extraction\ Buffer}$: the volume of the Extraction Buffer, 1 mL; V_{sample}: the volume of the supernatant in the reaction system, 0.01 mL; ε: NADPH molar extinction coefficient, 6.22×10^3 L/mol/cm; d: 96-well UV plate diameter, 0.5 cm; Cpr: protein concentration (mg/mL); T: reaction time, 3 min; W: sample weight, g; 2,000: total number of bacteria or cells , 20 million; 10^9: unit conversion factor, 1 mol=10^9 nmol.

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.

Recommended Products

Catalog No.	Product Name
KTB1023	CheKine™ Micro Citrate Synthase (CS) Activity Assay Kit
KTB1230	CheKine™ Micro Succinate Dehydrogenase (SDH) Activity Assay Kit
KTB1240	CheKine™ Micro α-Ketoglutarate Dehydrogenase (α-KGDH) 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.