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CheKine™ Micro UDP-Glucose Pyrophosphosphprylase (UGP) Activity Assay Kit

Cat #: KTB1344 Size: 48 T/96 T

	Micro UDP-Glucose Pyrophosphosphprylase (UGP) Activity Assay Kit		
REF	**Cat #:** KTB1344	LOT	**Lot #:** Refer to product label
	Applicable sample: Animal and Plant tissues, Cells, Plasma, Serum or other Liquid samples		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

UDP-glucose pyrophosphorylase (UGP, EC2.7.7.9) is widely distributed in nature. It catalyzes the activation of glucose before glycogen synthesis. UDP-glucose (UDPG) is synthesized from glucose-1-phosphate and UTP. UDPG is the main active enzyme form in higher plants and animals. As a glucose-based donor, it participates in the synthesis and metabolism of glycogen, sucrose, cellulose, etc. CheKine™ Micro UDP-Glucose Pyrophosphosphprylase (UGP) Activity Assay Kit can detect animal and plant tissues, cells, plasma, serum or other liquid samples. In this kit, UGP can catalyze the reversible formation of glucose-1-phosphate. NADP was transformed into NADPH by phosphoglucose mutase and 6-phosphoglucose dehydrogenase. UGP activity can be reflected by the change of 340 nm absorption value.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Reagent Ⅰ A	5 mL	10 mL	4℃, protected from light
Reagent Ⅰ B	1	1	-20℃, 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
Reagent Ⅴ	1 mL	2 mL	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 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℃.

Note: The Extraction Buffer has a pungent odor, so it is recommended to experiment in a fume hood.

Working Reagent Ⅰ: Prepared before use. Transfer Reagent Ⅰ B to Reagent Ⅰ A 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 Ⅱ: Prepared before use. Add 2 mL deionized water for 48 T and 2 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 Ⅲ: Prepared before use. Add 2 mL deionized water for 48 T and 2 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 2 mL deionized water for 48 T and 2 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 Ⅴ: 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 on ice. Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Cells: Collect 5×10^6 cells into the centrifuge tube, wash cells with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cells 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 10,000 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: 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. Operation table (The following operations are operated in the 96-well UV microplate or microquartz cuvette in turn):

Reagent	Blank Well (μL)	Test Well (μL)
Working Reagent Ⅰ	100	100
Reagent Ⅱ	20	20
Reagent Ⅲ	20	20
Reagent Ⅳ	20	20
Reagent Ⅴ	20	20

Deionized Water	20	0
Sample	0	20

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

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 UGP 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 UGP activity:

A. 96-well UV plates calculation formula as below

(1) Calculated by protein concentration

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

UGP (U/mg prot)=$[\Delta A\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(V_{Sample}\times Cpr)\div T$**$=321.54\times\Delta A\div Cpr$**

(2) Calculated by fresh weight of samples

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

UGP (U/g fresh weight)=$[\Delta A\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(W\times V_{Sample}\div V_{Total\ sample})\div T$**$=321.54\times\Delta A\div W$**

(3) Calculated by cell number

Active unit definition: The consumption of 1 nmol of NADP per 10^4 cell min was defined as one unit of enzyme activity.

UGP (U/10^4 cell) = $[\Delta A\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(n\times V_{Sample}\div V_{Total\ sample})\div T$**$=321.54\times\Delta A\div n$**

(4) Calculated by volume of samples

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

UGP (U/mL) = $[\Delta A\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(V_{Sample}\div V_{Total\ sample})\div T$**$=321.54\times\Delta A$**

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, 0.02 mL; $V_{Total\ sample}$: Extraction Buffer volume added, 1 mL; T: reaction time, 5 min; Cpr: sample protein concentration, mg/mL; W: weight of sample, g; n: Total number of 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.

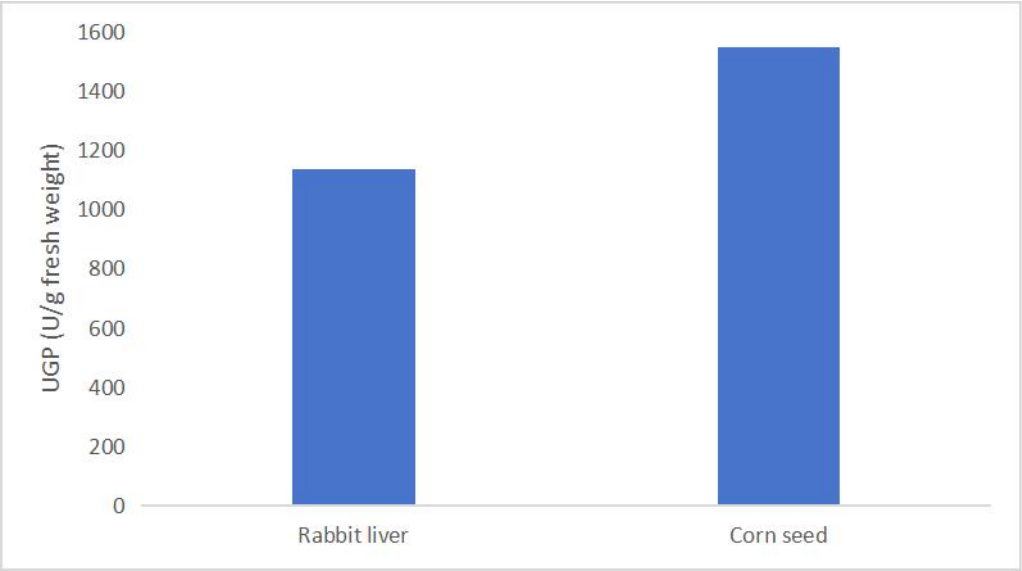

Figure 1. Determination UGP activity in rabbit liver and corn seed by this assay kit.

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.