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UDP-Glucose Pyrophosphosphprylase (UGP) Activity Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer

Cat No: AK0137-50T-48S

Size: 50T/48S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Sunlong staff in time.

Reagent	Size	Storage
Extract solution	Liquid 55 mL×1	2-8℃
Reagent I	Powder×1	-20℃
Reagent II	Powder×2	2-8℃
Reagent III	Powder×2	-20℃
Reagent IV	Powder×2	-20℃
Reagent V	Liquid 5 mL×1	2-8℃
Reagent VI	Liquid 15 mL×1	2-8℃

Solution preparation:

1. **Reagent I:** Add 30 mL distilled water to fully dissolve before use. The remaining reagents can be stored for 4 weeks at -20℃. Do not freeze and thaw repeatedly.

2. **Reagent II:** Add 2.5 mL distilled water to fully dissolve before use. The remaining reagents can be stored for 2 weeks at 4℃. Do not freeze and thaw repeatedly.

3. **Reagent III:** Add 0.7mL distilled water to fully dissolve before use. The remaining reagents can be stored for two weeks at -20℃. Do not freeze and thaw repeatedly. (This reagent is a freeze-dried reagent, there may be a large difference or even a small amount of macroscopic observation between different bottles, this phenomenon does not affect the use, the actual quality is the same)

4. **Reagent IV:** Add 1 mL distilled water to fully dissolve before use. The remaining reagents can be stored for two weeks at -20℃. Do not freeze and thaw repeatedly. Used up at one day. (This reagent is a freeze-dried reagent, there may be a large difference or even a small amount of macroscopic observation between different bottles, this phenomenon does not affect the use, the actual quality is the same)

5. **Working solution:** Calculate according to the amount required for the experiment before use, according to the ratio of Reagent I: Reagent II: Reagent III: Reagent IV: Reagent V: Reagent VI= 600: 100: 20: 40:100:250, mix well before use.

Product Description:

UDP-glucose pyrophosphorylase (UDP-glucose pyrophosphosphprylase, 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.

UGP can catalyze the reversible formation of glucose-1-phosphate. NADP is transformed into NADPH by phosphoglucose mutase and 6-phosphoglucose dehydrogenase. UGP activity can be reflected by the change of 340nm absorption value.

Required but Not Provided:

Ultraviolet spectrophotometer, desk centrifuge, water-bath, adjustable pipette, 1 mL quartz cuvette, mortar/homogenizer/cell ultrasonic crusher, ice and distilled water.

Protocol

I. Preparation:

1. Tissue: according to the ratio of mass (g): extraction volume (mL): 1:5-10 to add the extract. It is suggested that add 1 mL of extract to 0.1 g of tissue. Homogenate on ice. Centrifuge at 10000 g 4℃ for

   10 min. Take the supernatant on ice for test.

2. Bacteria and cells: according to the ratio of 10^4 cells: extract volume (mL) 500-1000:1. It is suggested to take about 500 million bacteria/cells and add 1 mL extraction reagent. Bacteria/cells is split by ultrasonication (power 300w, ultrasonic 3s, interval 7s, total time 3 min). Centrifuge at 10000 g 4℃ for

   10 min. Take the supernatant on ice for test.

3. Serum and other liquids: detect directly.

II. Determination procedure:

1. Preheat ultraviolet spectrophotometer for 30 min, adjust wavelength to 340 nm, set the counter to zero with distilled water.
2. Operation table:

Reagent (μL)	Test tube (A_T)	Blank tube (A_B)
Sample	100	
Working solution	900	900
Distilled water	-	100

Add the above reagents to the 1 mL quartz cuvette respectively. Mix thoroughly. Measure the absorbance of A1 at 340 nm for 10s. Then put it in a 37℃ water bath or incubator for 5 min. Take it out and dry it. Measure the absorbance of A2 at 340 nm for 310s. Calculate $\Delta A_T=A2_T-A1_T$, $\Delta A_B=A2_B-A1_B$, $\Delta A= \Delta A_T-\Delta A_B$. Blank tube only needs to be tested once or twice.

III. UGP Calculation:

1) Protein concentration:

Unit definition: One unit of enzyme is defined as the amount of enzyme that catalyzes the production of 1 nmol NADPH per minute every mg tissue protein in the reaction system.

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UGP (U/mg prot) =[ΔA÷(ε×d) ×V_T×10^9] ÷(Cpr ×V_{SA}) ÷T= 321.54×ΔA÷Cpr

2) Sample weight:

Unit definition: One unit of enzyme is defined as the amount of enzyme that catalyzes the production of 1 nmol NADPH per minute every g tissue weight in the reaction system.

UGP (U/g weight) =[ΔA÷(ε×d) ×V_T×10^9] ÷(W×V_{SA}÷V_E) ÷T= 321.54×ΔA÷W

3) Cells

Unit definition: One unit of enzyme is defined as the amount of enzyme that catalyzes the production of 1 nmol NADPH per minute every 10^4 cells in the reaction system.

UGP (U/10^4 cell) =[ΔA÷(ε×d) ×V_T×10^9] ÷(500×V_{SA}÷V_E) ÷T= 0.643×ΔA

4) Liquid volume

Unit definition: One unit of enzyme is defined as the amount of enzyme that catalyzes the production of 1 nmol NADPH per minute in 1 mL serum in the reaction system.

UGP (U/mL) =[ΔA÷(ε×d) ×V_T×10^9] ÷V_{SA}÷T= 321.54×ΔA

ε: NADPH molar extinction coefficient, 6.22×10^3 L/mol/cm;

d: Light path of cuvette, 1 cm;

10^9: Unit conversion coefficient, 1 mol = 10^9 nmol;

V_T: Total volume of reaction system, 1×10^{-3} L;

V_{SA}: Sample volume, 0.1 mL;

Cpr: Protein concentration, mg/mL;

W: Sample weight, g;

V_E: Extract solution volume of cells, 1 mL;

T: Reaction time, 5 min;

500: Total number of cells or bacteria, 5 million.

Note:

1. The blank tube is a test tube for testing the quality of each reagent component. Under normal conditions, the change does not exceed 0.01.

2. When the ΔA is greater than 0.6 or A2 is greater than 1.2, it is recommended to dilute the sample for determination. When the ΔA is less than 0.01, it is recommended that the reaction time can be prolonged (5 min or 10 min) for determination.

Related products:

AK0556/AK0555 β- 1,3-glucanase(β- 1,3-GA) Activity Assay Kit

AK0199/AK0198 Acidic Xylanase Activity Assay Kit