

UDP-Glucose Pyrophosphorylase (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

Size: 50T/48S

Components:

Extract solution: Liquid 55 mL×1, store at 4°C;

Reagent I: Powder×2, store at -20°C and protect from light; Add 15 mL distilled water to fully dissolve before use. The remaining reagents can be stored for two weeks at -20°C. Do not freeze and thaw repeatedly.

Reagent II: Powder×1, store at 4°C and protect from light; Add 5 mL distilled water to fully dissolve before use. The remaining reagents can be stored for one week at 4°C. Do not freeze and thaw repeatedly.

Reagent III: Powder×1, store at -20°C and protect from light; Add 1.4 mL distilled water to fully dissolve before use. The remaining reagents can be stored for two weeks at -20°C. Do not freeze and thaw repeatedly.

Reagent IV: Powder×2, store at -20°C and protect from light; Add 1 mL distilled water to fully dissolve before use. The remaining reagents can be stored for two weeks at -20°C. Do not freeze and thaw repeatedly.

Reagent V: Liquid 5 mL×1, store at 4°C;

Reagent VI: Liquid 15 mL×1, store at 4°C;

Product Description:

UDP-glucose pyrophosphorylase (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.

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 340nm absorption value.

Required but Not Provided:

Ultraviolet spectrophotometer, balance, low temperature desk centrifuge, water-bath, transferpettor, 1 mL quartz cuvette, EP tube, mortar/homogenizer, 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°C for 10 min. Take the supernatant on ice for test.
2. Bacteria and cells: according to the ratio of 10⁴ 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°C 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. Working solution: according to the volume ratio of reagent I, II, III, IV, V, VI=600: 100: 20: 40: 100: 250. Mix thoroughly. Prepare it when the solution will be used.
3. 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 37C water bath or incubator for 5 min. Take it out and dry it. Measure the absorbance of A₂ at 340 nm for 310s. Calculate $\Delta A_T = A_{2T} - A_{1T}$, $\Delta A_B = A_{2B} - A_{1B}$, $\Delta A = \Delta A_T - \Delta A_B$. 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.

$$\text{UGP (U/mg prot)} = [\Delta A \div (\epsilon \times d) \times V_T \times 10^9] \div (C_{pr} \times V_{SA}) \div T = 321.54 \times \Delta A \div C_{pr}$$

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.

$$\text{UGP (U/g weight)} = [\Delta A \div (\epsilon \times d) \times V_T \times 10^9] \div (W \times V_{SA} \div V_E) \div T = 321.54 \times \Delta A \div W$$

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 10⁴ cells in the reaction system.

$$\text{UGP (U/10}^4 \text{ cell)} = [\Delta A \div (\epsilon \times d) \times V_T \times 10^9] \div (500 \times V_{SA} \div V_E) \div T = 0.643 \times \Delta A$$

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.

$$\text{UGP (U/mL)} = [\Delta A \div (\epsilon \times d) \times V_T \times 10^9] \div V_{SA} \div T = 321.54 \times \Delta A$$

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;

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 A_2 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