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/microplate reader

Cat No: BC3365

Size: 100T/96S

Components:

Extract solution: Liquid 110 mL×1, store at 4℃;

Reagent I: Powder×1, store at -20℃ 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℃. Do not freeze and thaw repeatedly.

Reagent II: Powder×1, store at 4℃ and protect from light; Add 2.5 mL distilled water to fully dissolve before use. The remaining reagents can be stored for one week at 4℃. Do not freeze and thaw repeatedly.

Reagent III: Powder×1, store at -20℃ 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℃. Do not freeze and thaw repeatedly.

Reagent IV: Powder×1, store at -20℃ 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℃. Do not freeze and thaw repeatedly.

Reagent V: Liquid 2.5 mL×1, store at 4℃;

Reagent VI: Liquid 5 mL×1, store at 4℃;

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 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/microplate reader, balance, low temperature desk centrifuge, water-bath, transferpettor, micro quartz cuvette/96-well flat-bottom UV plate, 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℃ 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. 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	20	
Working solution	180	180
Distilled water	-	20
Add the above reagents to the micro quartz cuvette /96 well UV plate 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. If the microplate reader has the temperature control function, the temperature can be adjusted to 37℃. 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 test once or twice.		

III. UGP Calculation:

a. Micro quartz cuvette

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.

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

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) $=[\Delta A\div(\varepsilon\times d)\times V_T\times 10^9]\div(W\times V_{SA}\div V_E)\div T=321.54\times\Delta A\div W$

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 10^4 cells in the reaction system.

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

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.

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

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;

b. 96 well UV plate

The optical diameter d=1 cm of the cuvette in the above formula is changed to 0.6 cm of the 96 well UV plate.

Note:

1. The blank tube is a test hole 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 1.5, 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:

BC0360/BC0365 β-1,3-glucanase(β-1,3-GA) Activity Assay Kit

BC2600/BC2605 Acidic Xylanase Activity Assay Kit