

# 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:** BC3360

**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 10<sup>4</sup> 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 <sub>T</sub> ) | Blank tube (A <sub>B</sub> ) |
|------------------|-----------------------------|------------------------------|
| 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°C 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 10<sup>4</sup> 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.

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

$\epsilon$ : NADPH molar extinction coefficient,  $6.22 \times 10^3$  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,  $1 \times 10^{-3}$  L;

$V_{SA}$ : Sample volume, 0.1 mL;

C<sub>pr</sub>: 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  $\Delta A$  is greater than 0.6 or  $A_{2}$  is greater than 1.2, it is recommended to dilute the sample for determination. When the  $\Delta A$  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  $\beta$ -1,3-glucanase( $\beta$ -1,3-GA) Activity Assay Kit

BC2600/BC2605 Acidic Xylanase Activity Assay Kit