

## 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°C;

**Reagent I:** Powder×1, 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 4 weeks at -20°C. Do not freeze and thaw repeatedly.

**Reagent II:** Powder×2, store at 4°C and protect from light; Add 1.25 mL distilled water to fully dissolve before use. The remaining reagents can be stored for 2 weeks at 4°C. Do not freeze and thaw repeatedly.

**Reagent III:** Powder×1, store at -20°C and protect from light; Add 0.7 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 2.5 mL×1, store at 4°C;

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

**Working solution:** According to the number of test tubes, Reagent I : Reagent II : Reagent III : Reagent IV : Reagent V : Reagent VI is mixed with the volume ratio = 600 : 100 : 20 : 40 : 100 : 250. Used up at one day.

### 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°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	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 A<sub>1</sub> at 340 nm for 10s. Then put it in a 37°C-water bath or incubator for 5 min. If the microplate reader has the temperature control function, the temperature can be adjusted to 37°C. Take it out and dry it. Measure the absorbance of A<sub>2</sub> 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 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.

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

##### 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  $10^4$  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;

$C_{pr}$ : 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  $\Delta A$  is greater than 0.6 or  $A_{2}$  is greater than 1.5, 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

