

Pyrophosphate: Fructose 6-phosphate-1 Phosphotransferase Assay Kit

Note: Take two or three different samples for prediction before test.

Detection instrument: Spectrophotometer

Cat No: BC3400

Size: 50T/48S

Components:

Extract solution: 55mL × 1, stored at 4 °C.

Reagent 1: 40mL × 1, stored at 4 °C and protected from light.

Reagent 2: Powder × 1, stored at -20°C and protected from light. Just before use, add 6 mL of distilled water to fully dissolve. Unused reagents are stored at -20°C for 1 weeks after dispensing to avoid repeated freeze-thaw cycles.

Reagent 3: powder × 1, stored at -20°C and protected from light. Just before use, add 6 mL of distilled water to fully dissolve. Unused reagents are stored at -20°C after dispensing. Prohibition of repeated freeze-thaw cycles.

Reagent 4: 91μL × 2, stored at 4°C and protected from light. Just before use, add 0.209 mL of distilled water to fully dissolve. stored at 4°C for 1 weeks.

Reagent 5: Powder × 2, stored at -20°C and protected from light. Just before use, add 0.3 mL of distilled water to fully dissolve. Unused reagents are stored at -20 °C for 1 weeks after dispensing.

Reagent 6: 60 μL × 1, stored at 4 °C and protected from light. Just before use, add 0.6 mL of distilled water to fully dissolve. stored at 4°C for 1 weeks.

Product Description:

Pyrophosphate: Fructose-6-phosphate-1-phosphotransferase (PFP, EC2.7.1.90) is a cytosolic enzyme that is widely present in plant tissues and Catalyzes the phosphorylation of fructose-6-phosphate like phosphofructokinase. As a result, the single PEP catalytic reaction is a reversible reaction, and pyrophosphate is used instead of ATP, which plays an important role in carbon metabolism of photosynthesis.

PFP catalyzes the conversion of fructose 6-phosphate to fructose 1,6-diphosphate, which is converted to dihydroxyacetone phosphate by the action of aldolase and triose phosphate isomerase, and then catalyzed by α-phosphate glycerol dehydrogenase and NADH to form Glycerol 3-diphosphate and NAD. The change in absorbance at 340 nm reflects the level of PFP activity.

Required material

Low temperature centrifuge, spectrophotometer, water bath/constant temperature incubator, mortar/homogenizer, 1 mL quartz cuvette, transferpette, ice and distilled water, EP tube.

Procedure:

I. Sample Extraction:

1. Tissue sample:

According to the mass of the tissue (g): the volume of the extract solution (mL) is 1: 5 ~ 10. Suggested 0.1g of tissue with 1mL of extract solution. Fully grind on ice, centrifugated at 20000g and 4°C for 15 min. Supernatant is placed on ice for test.

2. Bacteria or cells:

According to the number of cells (10^4): the volume of the extract solution (mL) is 500 ~ 1000: 1. Suggest 5 million with 1mL of Extract Solution. Use ultrasonication to split bacteria or cells (power 300W, work time 3s, interval 7s, total time 3 min). centrifugated at 20000g and 4°C for 15 min. Supernatant is placed on ice for test.

3. Liquids: direct detection.

II. Determination procedure:

1 Preheat the spectrophotometer 30 min, adjust wavelength to 340 nm, set zero with distilled water.

2 Add reagents with the following list:

Reagent name (μL)	Test tube (T)	Blank tube (T)
Reagent 1	670	670
Reagent 2	100	100
Reagent 3	100	100
Reagent 4	10	10
Reagent 5	10	10
Reagent 6	10	10
Sample	100	-
Distilled water	-	100

After thorough mixing, measure the initial value A_1 at 340 nm and the absorbance A_2 at 30 minutes at 37°C in a 1 mL quartz cuvette, and record them as A_{1T} , A_{1B} , and A_{2T} , A_{2B} . Calculate $\Delta A = (A_{1T} - A_{2T}) - (A_{1B} - A_{2B})$.

Note: Reagents 1, 2, 3, 4, 5, and 6 can also be formulated into working fluids according to the proportions of the operation table, which is now prepared for use; The blank tubes need only be made 1-2 times.

III. Calculation of PFP activity:

1 Calculated by micro quartz cuvette

1) Calculated by protein concentration:

Unit definition: One unit of enzyme activity is defined as that 1 mg of tissue protein per minute consumes 1 nmol of NADH.

$$\text{PFP activity (U/mg prot)} = \Delta A \div (\epsilon \times d) \times V_{RT} \times 10^9 \div (V_S \times C_{pr}) \div T = 53.59 \times \Delta A \div C_{pr}$$

2) Calculated by sample weight

Unit definition: One unit of enzyme activity is defined as that 1g of tissue per minute consumes 1 nmol of NADH.

$$\text{PFP activity (U/g fresh weight)} = \Delta A \div (\epsilon \times d) \times V_{RT} \times 10^9 \div (W \times V_S \div V_E) \div T = 53.59 \times \Delta A \div W$$

3) Calculated by bacteria or cell amount:

Unit definition: One unit of enzyme activity is defined as that 10 thousand bacteria or cells per minute consumes 1 nmol of NADH.

$$\text{PFP activity (U/10}^4 \text{ cell)} = \Delta A \div (\epsilon \times d) \times V_{RT} \times 10^9 \div (V_S \times N \div V_E) \div T = 53.59 \times \Delta A \div N \quad (10^4)$$

4) Calculated by serum and other liquids:

Unit definition: One unit of enzyme activity is defined as that 1 mL of liquids per minute consumes 1 nmol of NADH.

$$\text{PFP activity (U/mL)} = \Delta A \div (\epsilon \times d) \times V_{RT} \times 10^9 \div V_S \div T = 53.59 \times \Delta A$$

V_{RT} : total volume of reaction system, 0.001 L;

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

d : cuvette light path, 1 cm;

V_S : added sample volume, 0.1 mL;

V_E : volume of extract solution added, 1 mL;

T : reaction time, 30 min;

C_{pr} : sample protein concentration, mg/mL;

W : sample mass, 0.1 g;

10^9 : conversion factor, 1 mol = 10^9 nmol

N : number of cell

2. Calculated by 96-well UV plate:

Modify $d = 1$ cm in the above formula to $d = 0.6$ cm (the light path of a 96-well plate) for calculation.

Note:

1. The number of samples should not be too large to avoid delaying the enzymatic reaction time.

Experimental examples:

1. Take 0.1 g of bean sprouts and add 1 mL of Extract solution for sample processing. After centrifugation to take the supernatant, proceed according to the determination procedure. Calculate $\Delta A = (A_{1T} - A_{2T}) - (A_{1B} - A_{2B}) = (1.041 - 0.963) - 0 = 0.078$. The enzyme activity is calculated according to the sample mass.

$$\text{PFP activity (U/g fresh weight)} = 53.59 \times \Delta A \div W = 41.8 \text{ U/g fresh weight.}$$

Related products:

BC0990/BC0995 Plant Chlorophyll Content Assay Kit

BC2210/BC2215 Glyceraldehyde-3-phosphate Dehydrogenase(GAPDH) Activity Assay Kit

BC4330/BC4335 Plant Carotenoid Content Assay Kit