Pyrophosphate: Fructose 6-phosphate-1 Phosphotransferase Assay Kit

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

Detection instrument: Spectrophotometer/ Microplate reader

Cat No: BC3405

Size: 100T/96S

Components:

Extract solution: 110 mL × 1, stored at 4 °C.

Reagent 1: 15mL × 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 2.5 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 2.5 mL of distilled water to fully dissolve. Unused reagents are stored at -20°C after dispensing. Prohibition of repeated freeze-thaw cycles.

Reagent 4: 91 μL × 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 × 1, 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: 30 μL × 1, stored at 4 °C and protected from light. Just before use, add 0.3 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 from Glycerol 3-diphosphate and NAD. The change in absorbance at 340 nm reflects the level of PFP activity.

Required material

Low temperture centrifuge, spectrophotometer/microplate reader, water bath/constant temperature incubator, mortar/homogenizer, micro quartz cuvette/96 well plate, transferpettor, 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℃ 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℃ for 15 min. Supernatant is placed on ice for test.

3. Liquids: direct detection.

II. Determination procedure:

1 Preheat the spectrophotometer/microplate reader 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)
Reagent 1	134
Reagent 2	20
Reagent 3	20
Reagent 4	2
Reagent 5	2
Reagent 6	2
Sample	20

After thorough mixing, measure the initial value A1 at 340 nm and the absorbance A2 at 30 minutes at 37°C in a micro quartz cuvette/96-well UV plate, and record them as $A1_T$, $A1_B$, and $A2_T$, $A2_B$. Calculate $\Delta A = (A1_T\text{-}A2_T)\text{-}(A1_B\text{-}A2_B)$.

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.

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

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.

PFP activity (U/g fresh weight) =ΔA÷（ε×d）×V_{RT}×10^9÷(W ×V_S÷V_E) ÷T=53.59×ΔA÷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.

PFP activity (U/10^4 cell) =ΔA÷（ε×d）×V_{RT}×10^9÷(V_S×N÷V_E) ÷T=53.59×ΔA÷N（10^4）

4) Calculated by liquids:

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

PFP activity (U/mL) =ΔA÷（ε×d）×V_{RT}×10^9÷V_S÷T=53.59×ΔA

V_{RT}: total volume of reaction system, 2 × 10^{-4} L;

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

d: cuvette light path, 1 cm;

V_S: added sample volume, 0.02 mL;

V_E: volume of extract solution added, 1 mL;

T: reaction time, 30 min;

Cpr: 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 = 1cm in the above formula to d-0.6cm (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 shepherd's purse and add 1 mL of Extract solution for sample processing. After centrifugation to take the supernatant, proceed according to the determination procedure. After determination with micro quartz cuvette, calculate ΔA = (A1T-A2T)-(A1B-A2B)=(0.9984-0.9385)-0=0.0599. The enzyme activity is calculated according to the sample mass.

   PFP activity (U/g fresh weight) =53.59×ΔA÷W=32.1 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