

Sucrose Phosphoric Acid Synthetase (SPS) Activity Assay Kit

Detection instrument: Spectrophotometer/microplate reader

Catalog Number: NA0579

Size: 100T/48S

Components:

Extract Solution: 50 mL ×1. Storage at 4°C.

Reagent I: 2.5 mL×1. Storage at -20°C.

Reagent II: powder 10 mg×1. Storage at 4°C. Add 1 mL of distilled water to form 10 mg/mL sucrose solution. Dilute to 500 µg/mL with distilled water when the solution will be used.

Reagent III: 2 mL ×1. Storage at 4°C.

Reagent IV: 25 mL×1. Storage at 4°C.

Reagent V: 10 mL×1. Storage at 4°C.

Product Description

Sucrose is not only an important photosynthetic product, but also a major transport material in plants. Moreover, it is one of the storage forms of carbohydrates. Sucrose phosphate synthase (SPS) takes fructose-6-phosphate as the receptor, the sucrose produced by the reaction forms sucrose phosphate under the action of sucrose phosphatase. Sucrose phosphate synthase-sucrose phosphatase system is generally regarded as the main route of sucrose synthesis.

Sucrose phosphate synthase catalyzes fructose-6-phosphate to form sucrose phosphoric acid. The reaction between sucrose and resorcinol can present color change, which has a characteristic absorption peak at 480 nm and the enzyme activity is proportional to the depth of color.

Reagents and Equipment Required but Not Provided

Spectrophotometer/microplate reader, water-bath, centrifuge, adjustable pipette, micro glass cuvette/96 well plate, mortar/homogenizer and ice.

Procedure

I. Sample Extraction:

The tissue mass (g): Extract solution volume (mL) is 1:5-10 (We recommend weigh about 0.1 g of tissue and add 1 mL of Extract solution). conduct ice bath homogenate. Centrifuge at 8000×g for 10 minutes at 4°C, take the supernatant and placed on the ice for test.

II. Determination procedure:

1. Preheat the spectrophotometer 30 minutes, adjust the wavelength to 480 nm and set zero with distilled water
2. Add reagents into 1.5 mL centrifuge tube with the following list:

| Reagent Name (µL) | Test tube (T) | Control tube (C) | Standard tube (S) | Blank tube (B) |
|-------------------|---------------|------------------|-------------------|----------------|
|-------------------|---------------|------------------|-------------------|----------------|

| | | | | |
|---|-----|-----|-----|-----|
| Sample | 10 | 10 | - | - |
| Distilled water | - | 45 | 45 | 45 |
| Reagent I | 45 | - | - | - |
| Reagent II | - | - | 10 | - |
| Blending, water bath for 10 minutes at 25°C. | | | | |
| Reagent III | 15 | 15 | 15 | 15 |
| Boil in boiling water bath for about 10 minutes (cover tightly to prevent water loss) and cool. | | | | |
| Reagent IV | 210 | 210 | 210 | 210 |
| Reagent V | 60 | 60 | 60 | 60 |

Mix thoroughly, react in water bath for 20 minutes at 80°C. After cooling, measure the absorption value of each tube at 480 nm.

Calculate $\Delta A_T = A_T - A_C$, $\Delta A_S = A_S - A_B$.

III. Calculation of SPS Activity Unit

1. Calculate by the concentration of protein

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 µg of sucrose per minute every milligram of tissue protein.

SPS activity(µg/min/mg prot)=($C_S \times V_1 \times \Delta A_T \div \Delta A_S$) \div ($V_1 \times C_{pr}$) \div T=50 \times $\Delta A_T \div \Delta A_S \div C_{pr}$

2. Calculate by the sample fresh weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 µg of sucrose per minute every gram of tissue.

SPS activity (µg/min/g fresh weight)=($C_S \times V_1 \times \Delta A_T \div \Delta A_S$) \div ($W \times V_1 \div V_2$) \div T=50 \times $\Delta A_T \div \Delta A_S \div W$

C_S : Concentration of standard tube, 500 µg /mL;

V_1 : Add the sample volume into the reaction system, 0.01 mL;

V_2 : Add the extraction liquid volume, 1 mL;

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

W : Sample fresh weight, g;

T : Reaction time, 10 minutes.

Note:

Try to complete the determination within 30 minutes.

References:

[1] Schrader S, Sauter J J. Seasonal changes of sucrose-phosphate synthase and sucrose synthase activities in poplar wood (*Populus canadensis* Moench 'robusta') and their possible role in carbohydrate metabolism[J]. Journal of Plant Physiology, 2002, 159(8): 833-843.

Related Products:

NA0823/NA0581 Sucrose Synthetase(SS) Activity Assay Kit

NA0694/NA0453 Plant Sucrose Content Assay Kit

NA0382/NA0381 Acid Invertase(AI) Activity Assay Kit

NA0582/NA0824 Neutral Invertase (NI) Activity Assay Kit
NA0318/NA0317 Sucrose Synthetase (SS, Cleavage Direction) Activity Assay Kit
NA0316/NA0315 Solid-Acid Invertase (B-AI) Activity Assay Kit