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Sucrose Phosphoric Acid Synthetase (SPS) Activity Assay Kit

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

Detection instrument: Spectrophotometer

Catalog Number: AK0534

Size: 50T/24S

Components:

Extract solution: 30 mL ×1. Storage at 4℃ .

Solution I: 5 mL×1. Storage at -20℃ .

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

Solution III: 5 mL ×1. Storage at 4℃ .

Solution IV: 40 mL×1. Storage at 4℃ .

Solution V: 10 mL×1. Storage at 4℃ .

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 480nm and the enzyme activity is proportional to the depth of color.

Reagents and Equipment Required but Not Provided

Spectrophotometer, water-bath, table centrifuge, adjustable pipette, 1 mL glass cuvette, mortar/homogenizer, ice and distilled water.

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℃, 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	30	30	-	-
Distilled water	-	150	150	180
Reagent I	150	-	-	-
Reagent II	-	-	30	-
Blending, water bath for 10 minutes at 25℃ .				
Reagent III	50	50	50	50
Boil in boiling water bath for about 10 minutes (cover tightly to prevent water loss) and cool.				
Reagent IV	700	700	700	700
Reagent V	200	200	200	200

Mix thoroughly, react in the water-bath for 20 minutes at 80℃ . After cooling, with distilled water to zero, 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 is defined as an enzyme activity that per minute per milligram of tissue protein catalyze to produce 1 μg of sucrose.

SPS activity (μg/min/mg prot) $=(C_S \times V1 \times \Delta A_T \div \Delta A_S) \div (V1 \times Cpr) \div T = 50 \times \Delta A_T \div \Delta A_S \div Cpr$

2. Calculate by the sample fresh weight

Unit definition: One unit is defined as an enzyme activity that per minute per gram of tissue catalyze to produce 1μg sucrose.

SPS activity (μg/min/g fresh weight) $= (C_S \times V1 \times \Delta A_T \div \Delta A_S) \div (W \times V1 \div V2) \div T = 50 \times \Delta A_T \div \Delta A_S \div W$

C_S : Standard tube concentration, 500 μg /mL;

V1: Add the sample volume into the reaction system, 0.03 mL;

V2: Add the extraction liquid volume, 1 mL;

Cpr: Sample protein concentration, mg/mL;

W: Sample fresh weight, g;

T: Reaction time, 10 minutes.

3. Try to complete the determination within 30 minutes.