

Sucrose Synthetase(SS) Activity Assay Kit

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

Operation Equipment: Spectrophotometer/microplate reader

Catalog Number: NA0581

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 prepare 10 mg/mL sucrose solution when the solution will be used. Then dilute it with distilled water to 500 µg/mL for use.

Reagent III: 2 mL ×1. Storage at 4°C.

Reagent IV: 25 mL×1. Storage at 4°C.

Reagent V: 6 mL×1. Storage at 4°C.

Product Description

Sucrose is the main form of transport of photosynthetic products from source (leaf, etc.) to "sink" organs. Sucrose Synthetase (SS, EC 2.4.1.13) catalyzes the synthesis of sucrose from free fructose and glucose in plants.

SS catalyzes the reaction between free fructose and glucose donor UDPG to generate sucrose, and the reaction between sucrose and resorcinol can show color changes. There is a characteristic absorption peak at 480 nm, the enzyme activity of SS is proportional to the color.

Reagents and Equipment Required but Not Provided

Centrifuge, water-bath, adjustable pipette, spectrophotometer/microplate reader, micro quartz cuvette/ 96 well flat-bottom plate, ice and mortar/homogenizer.

Procedure

I. Extraction of determination sample

The tissue mass (g), Extract solution volume (mL) is 1:5-10 (We suggest that 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. Measuring operation table

- Preheat spectrophotometer/microplate reader more than 30 min, adjust wavelength to 480 nm and set zero with distilled water.
- Sample determination (add the following reagents in sequence in the 1.5 mL EP tube):

Reagent Name (µL)	Test tube (T)	Control tube (C)	Standard tube (S)	Blank tube (B)
Sample	10	10	-	-

Distilled water	-	45	45	55
Reagent I	45	-	-	-
Reagent II	-	-	10	-
Mix well and incubate for 10 min at 25°C.				
Reagent III	15	15	15	15
Boil in the 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 well, incubate at 80°C bath for 20 minutes. Determine the absorbance of each tube at 480 nm after cooling. The standard tube and the blank tube are only one tube. Set a control tube to each test tube.

III. Calculation of SS vitality unit

1) Calculate by protein concentration:

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.

$$\text{SS activity (U/mg prot)} = \{C_S \times V_1 \times (A_T - A_C) \div (A_S - A_B)\} \div (V_1 \times C_{pr}) \div T = 50 \times (A_T - A_C) \div (A_S - A_B) \div C_{pr}$$

2) Calculate by 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.

$$\text{SS activity (U/g fresh weight)} = \{C_S \times V_1 \times (A_T - A_C) \div (A_S - A_B)\} \div (W \times V_1 \div V_2) \div T = 50 \times (A_T - A_C) \div (A_S - A_B) \div W$$

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 extract solution volume, 1 mL;

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

W : Sample fresh weight, g;

T : Reaction time: 10 minutes.

3) Try to complete the determination within 30 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.

[2] Nomura T, Akazawa T. Enzymic mechanism of starch synthesis in ripening rice grains: VII. Purification and enzymic properties of sucrose synthetase[J]. *Archives of biochemistry and biophysics*, 1973, 156(2): 644-652.

[3] Pressey R., Potato sucrose synthetase: purification, properties, and changes in activity associated with maturation[J]. *Plant physiology*, 1969, 44(5): 759-764.

Related products:

- NA0821/NA0579 Sucrose Phosphoric Acid Synthetase(SPS) 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