Sucrose Synthase (SS-I, Breakdown Direction) Activity Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer/microplate reader

Cat No: NA0317

Size: 100T/48S

Components:

Extract solution: Liquid 60 mL×1, store at 4°C;

Reagent I: Liquid 8 mL×1, store at 4°C;

Reagent II: Powder×1, store at -20°C and protect from light. Add 2.5 mL Reagent I when the solution will be used. Mix thoroughly. The rest of reagent store at -20°C; Do not freeze and thaw repeatedly;

Reagent III: Liquid 8 mL×1, store at 4°C and protect from light;

Standard: Powder×1, 20 mg fructose. Store at 4°C. Add 1 mL distilled water when the solution will be used and make the final concentration is 20 mg/mL fructose solution for standby. Mix thoroughly. The rest of reagent store at 4°C for one week;

Product Description:

Sucrose synthetase (SS) is the key enzyme in the sugar metabolism of plants. It is responsible for the reversible reaction of sucrose decomposition and synthesis. Its decomposition activity can catalyze the hydrolysis of sucrose to UDPG and fructose. It is also involved in the synthesis of starch, cellulose and hemicellulose.

SS-I can catalyze the production of fructose and UDPG from sucrose and UDP. Fructose reacts with 3,5-Dinitrosalicylic acid to form brownish red substance with characteristic absorption peak at 540 nm. The activity of SS-I can be calculated by measuring the change of absorption value at 540 nm.

Required but Not Provided:

Spectrophotometer/microplate reader, desk centrifuge, water-bath, transferpettor, micro glass cuvette/96 well flat-bottom plate, mortar/homogenizer, EP tube, ice and distilled water.

Protocol

I. Preparation:

Tissue: according to the ratio of mass (g): extraction volume (mL): 1:5-10 to add the extract solution. It is suggested that add 1 mL of extract solution to 0.1 g of tissue. Homogenize on ice. Centrifuge at 8000 g 4℃ for 10 min. Take the supernatant on ice for test.

II. Determination procedure:

1. Preheat spectrophotometer/microplate reader for 30 min, adjust wavelength to 540 nm, set the counter to zero with distilled water.
2. Dilute 20 mg/mL standard solution with distilled water to 8, 6, 5, 4, 3, 2, 1 mg/mL standard solution for

standby.

3. Operation table:

Reagent (μL)	Control tube (A_C)	Test tube (A_T)	Standard tnbe (A_S)	Blank tube (A_B)
Sample	10	10	-	-
Standard	-	-	10	-
Distilled water	-	-	-	10
Reagent I	40		40	40
Reagent II	-	40	-	-
Mix thoroughly. Put it in 30℃-water bath for 30 min, and in 95℃ water bath for 10 min (cover tightly to prevent water loss).				
Reagent III	50	50	50	50
Mix thoroughly. Put it in boiling water for 5 min (cover tightly to prevent water loss). Cool to room temperature.				
Distilled water	400	400	400	400
Mix thoroughly. Add 200 μL reaction solution to the micro glass cuvette/96 well flat-bottom plate. Determine the absorption value A at 540 nm. Record as, $A_{C,}$ $A_{T,}$ A_S, A_B. $\Delta A=A_T-A_C$. $\Delta A_S=A_S-A_B$. Each test tube should be provided with a contrast tube. The standard curve only needs to test once.				

III. SS-I Calculation:

1. Standard curve

Take the concentration of each standard solution as x-axis, and the corresponding ΔA standard is y-axis. Then the linear regression equation y＝kx+b is obtained. Bring ΔA into the equation to get x (mg/mL).

2. Calculation

1) Protein concentration:

Unit definition: One unit of enzyme is defined as the amount of enzyme that catalyzes the decomposition the consumes of 1 μg fructose per minute every mg tissue protein in the reaction system.

SS-I (U/mg prot) = $x\times V_{SA}\div(Cpr\times V_{SA})\div T\times 10^3= 33.33x\div Cpr$

2) Sample weight:

Unit definition: One unit of enzyme is defined as the amount of enzyme that catalyzes the decomposition the consumes of 1 μg fructose per minute every gram tissue weight in the reaction system.

SS-I (U/g weight) $=x\times V_{SA}\div W\div T\times 10^3=33.33x\div W$

V_{SA}: Extract solution volume of cells, 1 mL;

10^3: Unit conversion factor, 1 mg = 10^3 μg;

T: Reaction time, 30 min;

Cpr: Protein concentration, mg/mL;

W: Sample weight, g.

Note:

1. When the A or ΔA is greater than 1.5, it is recommended to measure after dilution. The dilution ratio needs to be multiplied in the calculation formula.
2. Cover the EP tube tightly at 95°C water bath to prevent water loss. It needs to be cooled to room temperature before the next operation. It is to avoid splashing and scalding of liquid and to avoid affecting the test data.

Experimental examples:

1. Take 0.1 g of ryegrass and add 1 mL of Extract solution for sample processing. Follow the measurement procedure after taking the supernatant. After determination with 96 well flat-bottom plate, calculate $\Delta A=A_T-A_C=0.408-0.111=0.297$. Bring the result into the standard curve y=0.1231x-0.0365, and calculate x=2.7092. The enzyme activity is calculated according to the sample mass.

SS-I (U/g weight) =33.33x÷W=902.976 U/g weight.

Related products:

NA0823/NA0581 Sucrose Synthetase(SS) Activity Assay Kit
NA0821/NA0579 Sucrose Phosphoric Acid Synthetase(SPS) Activity Assay
NA0582/NA0824 Neutral Invertase(NI) Activity Assay Kit
NA0694/NA0453 Plant Sucrose Content Assay Kit