Plant Sucrase Activity Assay Kit

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

Operation Equipment: Spectrophotometer/ Microplate Reader

Cat No: BC0135

Size:100T/48S

Components:

Extract solution: Liquid 60 mL×1. Storage at 4℃.

Reagent I: Liquid 2 mL×1. Storage at 4℃.

Reagent II: Powder×1. Storage at 4℃. Add 1 mL of distilled water before use. Unused reagent can be stored for one week at 4℃.

Reagent III: Liquid 4 mL×1. Storage at room temperature.

Standard: Powder×1. Storage at 4℃. Dissolve the standard with 1 mL of distilled water to generate a 10 mg/mL glucose solution standard. Unused reagent can be stored for one week at 4℃.

Product Description:

Sucrase is one of the key enzymes in carbohydrate digestion and absorption. It can hydrolyzes sucrose to produce corresponding monosaccharides which are absorbed by the body.

3.5-Dinitrosalicylic acid is reduced to brown-red amino compound by co-heating with reducing sugar. The absorbance ratio of brown-red amino compound is in direct proportion to the contents of reducing sugar. This product uses the 3.5-dinitrosalicylic acid method to determine the content of reducing sugars produced by plant sucrase catalyzing sucrose degradation, then the hydrolysis rate of plant sucrase can be obtained.

Reagents and Equipment Required but Not Provided:

Microplate reader/spectrophotometer, water bath, refrigerated centrifuge, adjustable transferpettor, micro glass cuvette/96 well flat-bottom plate, mortar/homogenizer, ice and distilled water.

Procedure:

I. Sample preparation:

According to sample weight (g): extract solution (mL) is 1:5~10 to extract. Add 1 mL of extraction reagent to 0.1 g of tissue, and fully homogenized on ice bath. Centrifuge at 8000×g for 10 minutes at 4℃ to remove insoluble materials, and take the supernatant on ice before testing.

II. Determination procedure:

1) Preheat microplate reader or spectrophotometer for 30 minutes, adjust the wavelength to 540 nm, set zero with distilled water.

2) Standard: Dilute the 10 mg/mL standard solution to 2.5, 2, 1.5, 1, 0.8, 0.6, 0.4, 0.2, 0 mg/mL (0 mg/mL is blank tube , abbreviated as B) with distilled water.

3) Add the following reagents in 1.5 mL EP tubes:

Reagent	Contrast tube (C)	Test tube (T)	Standard tube (S)
Reagent I ((μL)	15	15	15
Distilled water (μL)	15	-	-
Sample (μL)	30	30	-
Standard solution (μL)	-	-	30
Reagent II (μL)	-	15	15
Mix thoroughly and incubate accurately at 25℃ water bath for 10 minutes.			
Reagent III (μL)	30	30	30
Mix thoroughly, then place the tubes in a boiling water bath for 10 minutes(cover tightly to prevent moisture loss) and rapid cooling by ice bath.			
Distilled water (μL)	210	210	210
Mix thoroughly. Take 200 μL to micro glass cuvette or 96 well flat-bottom plate and detect the absorbance at 540 nm, record as A_C, A_T and A_S respectively. Each test tube requires a contrast tube.$\Delta A_T=(A_T-A_C)$, $\Delta A_S=(A_S-A_B)$.			

III. Calculation:

1. Standard curve

The concentration of standard solution as x-axis, ΔA_S as y-axis, obtain the equation y=kx+b. Take ΔA_T to the equation to acquire x (mg/mL) value.

2. Calculation

1) Tissue protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the hydrolyzation of 1 μg of sucrose in the reaction system per minute every milligram protein.

Plant Sucrase Activity (U/mg prot)=(1000×x×V1)÷(V1×Cpr)÷T=100×x÷Cpr

2) Tissue weight

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

Plant Sucrase Activity (U/g fresh weight)=(1000×x×V1)÷(W÷V2×V1)÷T=100×x÷W

1000: 1 mg/mL=1000 μg/mL;

V1: Sample volume (mL), 0.03 mL;

V2: Extract solution volume, 1 mL;

Cpr: Supernatant sample protein concentration, mg/mL;

T: Reaction time (min), 10 minutes;

W: Sample weight, g.

Note:

If A>1.2, the sample can be determined after being appropriately diluted with extract solution.

References：

[1] Karley A J, Ashford D A, Minto L M, et al. The significance of gut sucrase activity for osmoregulation in the pea aphid, Acyrthosiphon pisum[J]. Journal of insect physiology, 2005, 51(12): 1313-1319.

Related Products：

BC0580/BC0585	Sucrose Synthetase (SS) Activity Assay Kit
BC0600/BC0605	Sucrose Phosphoric Acid Synthetase(SPS) Activity Assay Kit
BC0560/BC0565	Acid Invertase(AI) Activity Assay Kit
BC0570/BC0575	Neutral Invertase(NI) Activity Assay Kit
BC2460/BC2465	Plant Sucrose Content Assay Kit
BC4310/BC4315	Sucrose Synthetase (SS-I, Cleavage Direction) Activity Assay Kit