Plant Sucrase 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: BC0135

Size: 100T/48S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation Condition
Extract solution	Liquid 60 mL×1	2-8℃
Reagent I	Liquid 2 mL×1	2-8℃
Reagent II	Powder ×1	2-8℃
Reagent III	Liquid 4 mL×1	RT
Standard	Powder ×1	2-8℃

Solution Preparation:

1. Reagent II: Add 1 mL of distilled water before use. The left reagent can be stored at 2-8°C for one week.
2. Standard: Dissolve with 1 mL of distilled water to generate a 10mg/mL glucose solution standard. Unused reagent can be stored for one week at 2-8℃.

Product Description:

Sucrase (EC 3.2.1.26) is one of the key enzymes in carbohydrate digestion and absorption. It can hydrolyze 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 volume (mL) is 1:5~10 to extract. It is recommended that add 1 mL of Extract solution 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/spectrophotometer for 30 minutes, adjust the wavelength to 540 nm, set spectrophotometer to 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 (wrap the sealing film to prevent bursting) and rapid cooling by ice bath.			
Distilled water (μL)	210	210	210
Mix thoroughly. Take 200 μL to micro glass cuvette/96 well flat-bottom plate and detect the absorbance at 540 nm, record as A_C, A_T and A_S respectively. $\Delta A_T=A_T-A_C$, $\Delta A_S=A_S-A_B$. The blank tube and standard curve only need to be measured 1-2 times. A contrast tube is required for each test tube.			

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 value (mg/mL).

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 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.

Recent Product Citations:

[1] Yu H, Qi W, Cao X, Hu J, Li Y, Peng J, Hu C, Qu J. Microplastic residues in wetland ecosystems: Do they truly threaten the plant-microbe-soil system? Environ Int. 2021 Nov; 156:106708. doi: 10.1016/j.envint.2021.106708. Epub 2021 Jun 18. PMID: 34153891.

[2] Chai G, Wang D, Shan J, Jiang C, Yang Z, Liu E, Meng H, Wang H, Wang Z, Qin L, Xi J, Ma Y, Li H, Qian Y, Li J, Lin Y. Accumulation of high-molecular-weight polycyclic aromatic hydrocarbon impacted the performance and microbial ecology of bioretention systems. Chemosphere. 2022 Jul; 298:134314. doi: 10.1016/j.chemosphere.2022. 134314. Epub 2022 Mar 12. PMID: 35292274.

[3] Chen W, Li S, Bai D, Li Z, Liu H, Bai L, Pan L. Detoxification mechanism of herbicide in Polypogon fugax and its influence on rhizosphere enzyme activities. Ecotoxicol Environ Saf. 2023 Sep 15; 263:115263. doi: 10.1016/j.ecoenv. 2023.115263. Epub 2023 Jul 18. PMID: 37473705.

[4] Zhou J, Liu H, Wu H, Wang X, Shen Y, Ren A, Tian S, Ma Y. Field tests of crop growth using hydrothermal and spray-dried cephalosporin mycelia dregs as amendments: Utilization of nutrient and soil antibiotic resistome. Environ Res. 2021 Nov; 202:111638. doi: 10.1016/j.envres.2021.111638. Epub 2021 Jul 15. PMID: 34273368.

[5] Yang M, Huang DY, Tian YB, Zhu QH, Zhang Q, Zhu HH, Xu C. Influences of different source microplastics with different particle sizes and application rates on soil properties and growth of Chinese cabbage (Brassica chinensis L.). Ecotoxicol Environ Saf. 2021 Oct 1; 222:112480. doi: 10.1016/j.ecoenv.2021.112480. Epub 2021 Jun 30. PMID: 34217116.

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.

[2] Iwona M, Maciej S, Łukasz M. et al. Changes in carbohydrate and isoflavonoid metabolism in yellow lupine in response to infection by Fusarium oxysporum during the stages of seed germination and early seedling growth [J]. Physiological and Molecular Plant Pathology, 2010, 75: 46-55.

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

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