Acid Invertase (AI) Activity Assay Kit

Note: Before the experiment, it is recommended to select 2-3 sample with large expected differences for pre-experiment.

Operation Equipment: Spectrophotometer/Microplate reader

Catalog Number: BC0565

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	Storage
Extract Solution	Solution 60 mL×1	2-8℃
Reagent I	Solution 30 mL×1	2-8℃
Reagent II	Powder×1	2-8℃
Reagent III	Solution 15 mL×1	2-8℃
Standard	Powder×1	2-8℃

Solution preparation:

1. Reagent II: Add 15 mL of Reagent I to fully dissolve for standby when the solution will be used. Unused reagents stored at 2-8 ℃ for 4 weeks.
2. Standard solution: 10 mg of glucose. Add 1 mL of distilled water with filly dissolve before use to prepare 10 mg/mL glucose standard solution for standby, stored at 2-8 ℃ for 4 weeks.

Product Description

Invertase (Ivr) catalyzes the irreversible decomposition of sucrose into fructose and glucose, which is one of the key enzymes in sucrose metabolism of higher plants. According to the optimal pH, Ivr can be divided into two types: acid invertase (AI) and neutral invertase (NI). AI (EC 3.2.1.26) mainly exists in cell vacuole or free space, and the optimal pH is 4.5-5.0 (acid). It can regulate the utilization of sucrose in vacuole and the accumulation of sugar in fruit by degrading sucrose in vacuole.

AI catalyzes the degradation of sucrose to produce reducing sugar, and further reacts with 3,5-dinitrosalicylic acid to form brownish red amino compound, which has a characteristic light absorption at 540 nm. the increase rate of light absorption at 540 nm in a certain range is in direct proportion to AI activity.

Reagents and Equipment Required but Not Provided

Spectrophotometer/microplate reader, desktop centrifuge, water-bath/constant temperature incubator, adjustable pipette, micro glass cuvette/ 96 well flat-bottom plate, mortar/homogenizer, ice and distilled water.

Procedure

I. Sample preparation:

Weigh about 0.1 g of tissue, add 1 mL of Extract solution for ice bath homogenization. Centrifuge at 12000 ×g for 10 minutes at 4℃, take the supernatant and place it on ice for test.

II. Determination steps:

1. Preheat spectrophotometer/microplate reader more than 30 minutes, adjust wavelength to 540 nm and set spectrophotometer to zero with distilled water.
2. Dilute the standard solution to 2.0, 1.5, 1.0, 0.5, 0.25 and 0 mg/mL of glucose standard solution (0mg/mL is blank tube).
3. Preheat some Reagent I and Reagent II at 37 ℃ for more than 10 minutes.
4. Standard dilution table:

Serial Number	Pre dilution concentration (mg/mL)	Standard solution volume (μL)	Distilled water volume (μL)	Diluted concentration (mg/mL)
1	10	200	800	2
2	2	600	200	1.5
3	2	200	200	1
4	1	200	200	0.5
5	0.5	200	200	0.25
6	-	-	400	0 (Blank tube)

Note: Each standard tube in the following experiment requires 50μL of standard solution volume (be careful not to directly test the absorbance value in this step).

5. Operation table (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)
Sample	50	50	-
Reagent I	-	200	-
Reagent II	200	-	200
Standard solution	-	-	50
Mix well. After 30 minutes of accurate water bath at 37℃, boil for about 10 minutes (Wrap the sealing film to prevent bursting). After water cooling, mix well (to ensure constant concentration). Centrifuge at 12000 ×g for 5 minutes at 4℃ and take the supernatant.			
Supernatant	200	200	200
Reagent III	125	125	125
Mix well and boil for about 10 minutes (wrap with sealing film to prevent bursting). After cooling with running water, mix thoroughly. Take 200 μL and transfer it to a 96 well plate or micro glass cuvette. Measure the absorbance values of each tube at 540nm, which are recorded as A_T, A_C, A_S, and A_B (0 mg/mL). $\Delta A_T=A_T - A_C$, $\Delta A_S=A_S - A_B$. The standard curve only needs to be done 1-2 times.			

III. Calculation of AI activity:

1. Establishment of standard curve:

Plot the standard curve y=kx+b with glucose standard concentration on the x-axis and Δ A standard on the y-axis. According to the standard curve, substitute Δ A into the equation to obtain x (mg/mL).

2. Calculation of AI activity:

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 reducing sugar per minute at 37°C every milligram of protein.

AI activity(U/mg prot) = (x×Vs×1000)÷(Vs×Cpr)÷T×F=33.3×x÷Cpr×F

2) Calculate by sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 μg of reducing sugar per minute at 37°C every gram of tissue.

AI activity (U/g weight) = (x×Vs×1000)÷(W×Vs÷Ve)÷T×F =33.3×x÷W×F

1000: Unit conversion factor, 1 mg/mL = 1000 μg/mL;

Vs: The volume of sample added into the reaction system, 0.05 mL;

Ve: Add the volume of extract solution, 1 mL;

Cpr: Concentration of sample protein, mg/mL;

W: Sample weight, g;

T: Reaction time: 30 minutes.

F: Dilution ratio.

Note

1. If Reagent III is added and there is turbidity after boiling for 10 minutes, it is recommended to remove the precipitate by centrifugation and take the supernatant to determine the absorbance.
2. If ΔA is greater than 1.8, the sample can be measured after diluted with distilled water (multiply the corresponding dilution times in the calculation formula).
3. Because the Extract solution contains a certain concentration of protein (about 1mg/mL), the protein content of the Extract solution itself needs to be subtracted when determining the protein concentration of the sample.

Experimental example:

1. Take 0.1g of Hibiscus syriacus and add 1 mL of Extract solution for homogenization and grinding, take the supernatant and dilute it twice with distilled water, and then operate according to the determination steps. Use 96 well plate to calculate: ΔA_T = 0.574, ΔA_C = 0.378, ΔA =A_T- A_C = 0.574-0.378 = 0.196, bring in the standard curve y = 0.7408x - 0.0289, calculate x = 0.304

AI activity (U/g weight) = 33.3 × x ÷ W × dilution ratio = 33.3×0.304÷0.1×2=202.46 U/g weight.

References:

[1] Huang Y W, Nie Y X, Wan Y Y, et al. Exogenous glucose regulates activities of antioxidant enzyme, soluble acid invertase and neutral invertase and alleviates dehydration stress of cucumber seedlings[J]. Scientia horticulturae, 2013, 162: 20-30.

Related Products:

BC0570/BC0575	Neutral Invertase(NI) Activity Assay Kit
BC0580/BC0585	Sucrose Synthetase(SS) Activity Assay Kit
BC0600/BC0605	Sucrose Phosphoric Acid Synthetase(SPS) Activity Assay Kit
BC2460/BC2465	Plant Sucrose Content Assay Kit