Neutral Invertase (NI) 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: BC0575

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 reagent is still stored at 4℃ for 4 weeks.
2. Standard solution: 10 mg of anhydrous glucose. Add 1 mL of distilled water with filly dissolve before use to prepare 10 mg/mL glucose standard solution for standby, stored at 4℃ 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.

NI 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 NI activity.

Reagents and Equipment Required but Not Provided

Spectrophotometer/microplate reader, desktop centrifuge, water-bath, adjustable pipette, micro glass cuvette/ 96 well 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 more than 30 minutes, adjust wavelength to 540 nm and set zero with distilled water.
2. Preheat some Reagent I and Reagent II at 37 ℃ for more than 10 minutes.
3. Dilute the standard solution to 2, 1.5, 1, 0.5, 0.25 and 0 mg/mL of glucose standard solution (0mg/mL is blank tube).
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	2.0	600	200	1.5
3	2.0	200	200	1
4	1.0	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 200μL 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	-
Standard solution	-	-	50
Reagent I	-	200	-
Reagent II	200	-	200
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. and 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 NI 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 ΔA into the equation to obtain x (mg/mL).

2. Calculation of NI 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.

NI 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.

NI 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 1.6, 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 0.1066g of mango fruit tissue, process according to the sample extraction steps, dilute the supernatant by 8 times, follow the measurement steps, use a 96 well plate to measure the absorbance of the reaction solution at 540nm, calculate Δ A=A_T - A_C=0.282-0.252=0.030, substitute ΔA into the standard equation y=0.8528x-0.2786, R^2=0.9954， x=0.362 mg/mL.

Calculate the activity based on sample weight:

NI activity (U/g weight) =33.3 × x ÷ W × F=904.66 U/g weight.

2. Take 0.05g yeast powder and process it according to the sample extraction steps. Dilute thesupernatant 64 times and follow the measurement steps. Use a 96 well plate to measure the absorbance of the

reaction solution at 540nm. Calculate $\Delta A=A_T-A_C=1.265-0.075=1.190$, and substitute ΔA into the standard formula y=0.8528x-0.2786, $R^2=0.9954$，x=1.722 mg/mL， Calculate the activity based on sample quality:

NI activity (U/g weight) = 33.3 × x ÷ W × F=7.34×10^4 U/g weight.

Recent Product Citations：

[1] Ma L, Liang C, Cui Y, Du H, Liu H, Zhu L, Yu Y, Lu C, Benjakul S, Brennan C, Brennan MA. Prediction of banana maturity based on the sweetness and color values of different segments during ripening. Curr Res Food Sci. 2022 Sep 6;5:1808-1817. doi: 10.1016/j.crfs.2022.08.024. PMID: 36254243; PMCID: PMC9568694.

[2] Han W, Wang Y, Li H, Diao S, Suo Y, Li T, Sun P, Li F, Fu J. Transcriptome and Metabolome Reveal Distinct Sugar Accumulation Pattern between PCNA and PCA Mature Persimmon Fruit. Int J Mol Sci. 2023 May 11;24(10):8599. doi: 10.3390/ijms24108599. PMID: 37239943; PMCID: PMC10217969.

[3] Shi Y, Zhao Y, Yao Q, Liu F, Li X, Jin X, Zhang Y, Ahammed GJ. Comparative Physiological and Transcriptomic Analyses Reveal Mechanisms of Exogenous Spermidine-Induced Tolerance to Low-Iron Stress in Solanum lycopersicum L. Antioxidants (Basel). 2022 Jun 27;11(7):1260. doi: 10.3390/antiox11071260. PMID: 35883751; PMCID: PMC9312307.

[4] Gao L, Wang W, Xu C, Han X, Li Y, Liu Y, Qi H. Physiological and Transcriptomic Analyses Reveal the Effects of Elevated Root-Zone CO2 on the Metabolism of Sugars and Starch in the Roots of Oriental Melon Seedlings. Int J Mol Sci. 2022 Oct 19;23(20):12537. doi: 10.3390/ijms232012537. PMID: 36293393; PMCID: PMC9604077.

[5] Wu J, Chen H, Chen W, Zhong Q, Zhang M, Chen W. Effect of ultrasonic treatment on the activity of sugar metabolism relative enzymes and quality of coconut water. Ultrason Sonochem. 2021 Nov;79:105780. doi: 10.1016/j.ultsonch.2021.105780. Epub 2021 Oct 6. PMID: 34628309; PMCID: PMC8501503.

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:

BC0560/BC0565	Acid Invertase(AI) 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