Neutral Invertase (NI) Activity Assay Kit

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

Operation Equipment: Spectrophotometer/Microplate reader

Catalog Number: AK0284

Size: 100T/48S

Components:

Extract solution:100 mL×1. Storage at 4℃ .

Reagent I: 40 mL×1. Storage at 4℃ .

Reagent II: Powder×1. Storage at 4℃ . Add 20 mL of Reagent I to fully dissolve for standby when the solution will be used. Unused reagent is still stored at 4℃ .

Reagent III: 20 mL×1. Storage at 4℃ .

Standard solution: Powder×1. 10 mg of anhydrous glucose. Storage at 4℃ . Add 1 mL of distilled water with filly dissolve before use to prepare 10 mg/mL glucose standard solution for standby.

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 acid invertase (AI) and neutral invertase (NI). NI mainly exists in the cytoplasm and is responsible for decomposing sucrose into fructose and glucose.

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, and the increase rate of light absorption at 540 nm in a certain range is in direct proportion to NI activity. The activity of NI is calculated by the increasing rate of light absorption.

Reagents and Equipment Required but Not Provided

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

Procedure

I. Extraction of crude enzyme

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

II. Determination steps and sample adding table:

a. Preheat spectrophotometer/microplate reader more than 30 min, adjust wavelength to 540 nm and set zero with distilled water.

b. Dilute the standard solution with distilled water to 2, 1.5, 1.0, 0.5 and 0.25 mg/mL of glucose standard solution.

SunLong Biotech Co.,LTD
Tel: 0086-571-56623320 Fax:0086-571-56623318
E-mail:sales@sunlongbiotech.com
www.sunlongbiotech.com

c. Operate according to the following table:

Reagent Name (μL)	Test tube (T)	Control tube (C)	Standard tube (S)
Crude enzyme	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 (cover tightly to prevent water loss). 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, boil for about 10 minutes (cover tightly to prevent water loss). After water cooling, mix well. Take 200 μL of the supernatant to a micro glass cuvette/96 well flat-bottom plate, record the absorption value A of each tube at 540 nm, calculate $\Delta A = A_T - A_C$.

IV. Calculation of NI activity:

1. Production of standard curve: Draw the standard curve with the absorbance value of each concentration minus the absorbance of the blank tube (concentration of 0 mg/mL) as the y-axis and the glucose concentration as the x-axis. Take ΔA into the equation to get x (μmol/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℃ every milligram of protein.

NI activity(U/mg) = $(x \times V1 \times 1000) \div (V1 \times Cpr) \div T = 33.3 \times x \div Cpr$

2) Calculate by sample fresh 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℃ every gram of tissue.

NI activity (U/g fresh weight) = $(x \times V1 \times 1000) \div (W \times V1 \div V2) \div T = 33.3 \times x \div W$

1000: Unit conversion factor, 1 mg/mL = 1000 μg/mL;
V1: The volume of sample added into the reaction system, 0.05 mL;
V2: Add the volume of Extract solution, 1 mL;
Cpr: Concentration of sample protein, mg/mL;
W: Sample fresh weight, g;
T: Reaction time: 30 minutes.

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 the absorbance value is greater than 1, 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 Ilex and add 1 mL of Extract solution for homogenization, take the supernatant and dilute it twice, and then operate according to the steps. Calculate with 96 well plate: A_T= 0.686, A_C = 0.599, ΔA = A_T - A_C = 0.686-0.599 = 0.087, and bring it into the standard curve: y = 0.7035x+0.0263, calculate x = (0.087-0.0263) ÷ 0.7035 = 0.086

Ni activity (U/g mass) = 33.3 × x ÷ W × dilution ratio = 33.3 × 0.086 ÷ 0.1 × 2 = 57.276 U/g mass.

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:

AK0287/AK0286 Acid Invertase(AI) Activity Assay Kit
AK0538/AK0537 Sucrose Synthetase(SS) Activity Assay Kit
AK0534/AK0533 Sucrose Phosphoric Acid Synthetase(SPS) Activity Assay Kit
AK0226/AK0224 Plant Sucrose Content Assay Kit