Soil β-Glucosidase Assay Kit (Microanalysis)

Description

β-Glucosidase (β-D-Glucosidase, S-β-GC, EC 3.2.1.21), also known as β-D-glucoside glucohydrolase, gentiobiose enzyme, cellobiase (CB or β-G), and amygdalase, belongs to the cellulase family. It is a key component of the cellulolytic enzyme system and catalyzes the hydrolysis of terminal non-reducing β-D-glucosidic bonds, releasing β-D-glucose and the corresponding aglycone. S-β-GC plays a significant physiological role in carbohydrate metabolism in soil microorganisms.

Detection Principle

S-β-GC catalyzes the hydrolysis of p-nitrophenyl-β-D-glucopyranoside to produce p-nitrophenol, which exhibits a characteristic absorption at 400 nm.

Specifications

Composition and Storage Conditions (100T/48S):

Component	Specification	Storage Condition
CB0146M-A	Toluene, 5 mL (self-provided)	4 °C
CB0146M-B	Powder × 2 vials	Store at -20 °C. Before use, add 7.5 mL distilled water to each vial and dissolve completely. Unused reagent can be aliquoted and stored at -20°C for 4 weeks
CB0146M-C	30 mL × 1	4 °C
CB0146M-D	20 mL × 1	4 °C
CB0146M-Standard	1 mL × 1 (5 mmol/L)	4 °C

Note: Before the formal assay, it is recommended to perform a preliminary test with 2-3 samples expected to have significant differences.

Instructions

I. Required Equipment & Materials:

Visible spectrophotometer/microplate reader, benchtop centrifuge, water bath, 30–50 mesh sieve, adjustable pipettes, micro glass cuvette/96-well plate, mortar, ice, toluene, and distilled water.

II. Sample Preparation:

Air-dry fresh soil samples naturally or dry in an oven at 37°C, then pass through a 30–50 mesh sieve.

III. Assay Procedure:

1. Preheat the spectrophotometer for at least 30 min, set the wavelength to 400 nm, and zero the spectrophotometer with distilled water.

2. Preparation of standard solutions: Before use, take 100 μL of standard solution and add it to 400 μL of Reagent C to obtain a 1 mmol/L standard solution (i.e., 1000 μmol/L). Then dilute with distilled water to prepare standard solutions at concentrations of 250, 200, 100, 50, 25, and 12.5 μmol/L.

3. Add the following reagents sequentially into EP tubes:

Reagent	Sample Tube	Control Tube	Standard Tube	Blank Tube
Air-dried soil (g)	0.02	0.02		
CB0146M-A (μL)	10	10		
Mix thoroughly to fully wet the soil sample, and let stand at room temperature for 15 min.				
CB0146M-B (μL)	130			
CB0146M-C (μL)	160	160		
Mix thoroughly, incubate in a 37°C water bath for 1 h, then place in boiling water for 5 min (cap tightly to prevent water loss), and cool under running water.				
CB0146M-B (μL)		130		
Mix thoroughly, centrifuge at 10,000 g for 10 min at room temperature, and collect the supernatant. Add the following reagents to EP tubes or a 96-well plate:				
Supernatant (μL)	70	70		
Standard solution (μL)			70	
Distilled water (μL)				70
CB0146M-D (μL)	130	130	130	130
Mix thoroughly, let stand at room temperature for 2 min, then measure absorbance A at 400 nm. $\Delta A_{sample} = A_{sample} - A_{control}$ $\Delta A_{standard} = A_{standard} - A_{blank}$				

Note: Each sample tube requires a corresponding control tube.

IV. Calculation of S-β-GC Activity

1.Standard Curve

Establish a standard curve based on standard concentration (x, μmol/L) and absorbance ΔA_standard (y).

Substitute ΔA_sample (y) into the standard curve to obtain sample concentration (x, μmol/L).

2. Enzyme Activity Calculation

Definition: One unit of enzyme activity is defined as the amount of enzyme that produces 1 μmol of p-nitrophenol per day per gram of soil sample.

S-β-GC activity (U/g soil) = $x \times V_{total} \div W \div T = 0.36\ x$

T: Reaction time, 1 h = 1/24 d

V_{total}: Total reaction volume, 3×10^{-4} L

W: Sample weight, 0.02 g

Precautions

1.This product is for scientific research use by professionals only. It must not be used for clinical diagnosis or treatment, food or drug applications, and must not be stored in residential environments.

2.For your safety and health, please wear a lab coat and disposable gloves during operation.