Soil β-Glucosidase Assay Kit

Cat#: orb1499906 (manual)

Size: 100T/48S

Microassay

Product composition and storage conditions:

No.	Specifications	Storage Conditions
orb1499906 - A	Toluene 5mL (Self-provided)	Store at 4°C
orb1499906 - B	Powder ×2	Store at -20 ° C; Add 7.5mL distilled water to each bottle fully dissolve before use, and the remaining reagent is still stored at -20℃;
orb1499906 - C	30mL ×1	Store at 4°C;
orb1499906 - D	20mL ×1	Store at 4°C;
orb1499906 - Standard	1mL ×1 (5 mmol/L)	Store at 4°C.

※Before the formal measurement, be sure to take 2-3 samples with large expected differences for predetermination.

Introduction:

Significance: B-D-Glucosidase (S-β- GC, EC3.2.1.21), also known as β-D- glucoside glucose hydrolase, gentian disaccharidase, cellodisaccharidase, cellobias, or β-G, and amygdalosidase. It belongs to cellulase and is an important component of the cellulolytic enzyme system. It can hydrolyze and bind to the terminal non-reductive β-D- glucose bond and release β-D- glucose and corresponding ligands. S-β- GC has important physiological functions in the carbohydrate metabolism of soil microorganisms.

Principle: S-β-GC catalyzes the formation of p-nitrophenol from p-nitrobenzene -β-D glucopyranoside, which is characterized by light absorption at 400 nm.

Own supplies:

Visible spectrophotometer/plate reader, table centrifuge, water bath, 30~50 mesh sieve, adjustable pipette, micro glass cuvette/96-well plate, mortar, ice, toluene and distilled water.

Sample Processing:

Fresh soil sample is naturally air-dried or 37°C oven is air-dried, passing 30~50 mesh sieve.

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Measurement steps:

1. Preheat the visible spectrophotometer/microplate reader for at least 30 minutes, adjust the wavelength to 400nm, set zero with distilled water.
2. Preparation of standard: Take 100 µL standard solution immediately before use, add it into 400 µL reagent C to obtain 1 mmol/L standard solution (i.e. 1000 µmol/L), and then dilute with distilled water to the concentration of standard solution to be used 500, 250, 100, 50, 25 and 12.5 µmol/L.
3. Add the following reagents in sequence to the EP tube:

Reagent name	Measuring tube	Control tube	Standard tube	Blank tube
Air-dried soil sample (g)	0.02	0.02		
orb1499906 – A (ul)	10	10		
Vibrate and mix well to make all soil samples wet and leave at room temperature for 15 min.				
orb1499906 – B (ul)	130			
orb1499906 – C (ul)	160	160		
Mix well, place in 37°C water bath for 1h, then boiling water bath for 5 min (cover tightly to prevent water loss), and cool down with running water.				
orb1499906 –B (ul)		130		
Fully mix, centrifuge for 10 min at 10000 g 25°C, take supernatant (Add the following reagent to the EP tube or 96well plate).				
Supernatant (ul)	70	70		
Standard (ul)			70	
Distilled water (ul)				70
orb1499906 – D (ul)	130	130	130	130
Mix well, leave it at room temperature for 2 minutes, measure the absorbance A at 400 nm, and calculate the ΔA=A measuring -A control, ∆A standard=A standard -A blank.				

Note: A control tube is provided for each measuring tube.

S-β-GC activity calculation:

1. Standard curve establishment:

A standard curve was established based on the concentration of the standard tube (x, µmol/L) and the absorbance ΔA standard (y, ΔA standard). The ΔA assay (y, ΔA assay) was brought into the formula to calculate the sample concentration (x, µmol/L) according to the standard curve.

2. Calculation of S-β-GC enzyme activity:

Definition of units: 1µmol p-nitrophenol per g soil samples per day is defined as an enzyme activity unit.

S-β-GC enzyme activity (U/g soil sample) = x × V total ÷W÷T=0.36 x

T: Reaction time, 1h=1/24d; V total: Total volume of reaction system: 3×10^{-4}L; W: Sample weight, 0.02 g.

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