

Soil α -Glucosidase (S- α -GC) Activity Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer/microplate reader

Cat No: BC3085

Size: 100T/48S

Components:

Reagent I: Toluene 0.5 mL \times 1, store at 4°C; **Self-provided reagent;**

Reagent II: Powder \times 2, store at -20°C. Add 5 mL distilled water when the solution will be used. Mix thoroughly. The rest of reagent store at -20°C;

Reagent III: Liquid 15 mL \times 1, store at 4°C;

Reagent IV: Liquid 30 mL \times 1, store at 4°C;

Standard solution: Liquid 1 mL \times 1, 5 mmol/L of p-nitrophenol solution.

Product Description:

S- α -GC can catalyze the hydrolysis of glycosidic bonds between aryl or hydrocarbon groups and glycosyl groups to form glucose. It is one of the important components of cellulolytic enzyme system. It has important physiological functions in carbohydrate metabolism of soil microorganisms.

S-a-GC can catalyze the formation of p-nitrophenol from p-nitrobenzene-a-d-glucoopyranoside. It has characteristic light absorption at 400 nm.

Required but Not Provided:

Spectrophotometer/microplate reader, desk centrifuge, constant temperature incubator/water-bath, transferpettor, micro glass cuvette/96 well flat-bottom plate, 50 mesh sieve (or smaller), **toluene** (express delivery is not allowed) and distilled water.

Protocol

I. Preparation:

The fresh soil sample is dried by natural air or air in a 37°C oven, and passes through 30-50 mesh sieve.

II. Determination procedure:

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

2. Standard : Take 20 μ L of 5mmol/L p-nitrophenol solution, add 980 μ L of distilled water, mix well, and make a 100 μ mol/L standard solution for use now. (In the experiment, each tube needs 100 μ L, in order to reduce the experiment error, so prepare a large volume)

3. Operation table:

Reagent (μ L)	Test tube (A_T)	Control tube (A_C)	Standard tube (A_S)	Blank tube (A_B)
Sample	0.02	0.02	-	-

Reagent I	5	5	-	-
Mix thoroughly. Moisten the soil sample and place it at room temperature for 15 min.				
Reagent II	80	-	-	-
Reagent III	100	100	-	-
Mix thoroughly. Water bath at 37°C for 1 h. Boiling water bath for 5 min (cover tightly to prevent water loss). Cool it with running water/ice bath.				
Reagent II	-	80	-	-
Centrifugate at 10000 rpm, 25°C for 10 min. Take the supernatant for test.				
Supernatant	100	100	-	-
Standard	-	-	100	-
Distilled water	-	-	-	100
Reagent IV	200	200	200	200

Mix thoroughly. Stand for 2 min at room temperature. Determine the absorption value A. Record as A_T , A_C , A_S , A_B . $\Delta A = A_T - A_C$. $\Delta A_S = A_S - A_B$. Each test tube is provided with a control tube. Blank tube just need test once or twice.

III. S- α -GC Calculation:

Unit definition: One unit of enzyme is defined as the amount of enzyme that catalyzes the production of 1 μmol p-nitrophenol per day every gram tissue weight in the reaction system.

$$S\text{-}\alpha\text{-GC (U/g weight)} = \Delta A \div (\Delta A_S \div C_S) \times V_{RT} \div W \div T = 0.444 \times \Delta A \div \Delta A_S \div W$$

V_{RT} : Total reaction volume, 1.85×10^{-4} L;

C_S : Concentration of standard solution, 100 $\mu\text{mol/L}$;

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

W: Sample weight, g.

Note:

1. If $\Delta A < 0.01$, the water bath time of 37°C can be prolonged. If $\Delta A > 1.5$, the supernatant can be diluted before test. Pay attention to the change of various factors in the final calculation.

Experimental Example:

1. Two tubes of 0.02g clover soil are taken as the test tube and control tube. According to the test steps, measured by 96 well plate: $\Delta A = A_T - A_C = 0.421 - 0.238 = 0.183$, $\Delta A_S = A_S - A_B = 0.473 - 0.047 = 0.426$.

S - α - GC activity (U/g soil sample) = $0.444 \times \Delta A \div \Delta A_S \div W = 0.444 \times 0.183 \div 0.426 \div 0.02 = 9.5366$ U/g soil sample.

2. Take two tubes of 0.02g forest soil sample, namely the test tube and the control tube, according to

the determination steps, use 96 well plate to measure and calculate $\Delta A = A_T - A_C = 0.374 - 0.225 = 0.149$, $\Delta A_S = A_S - A_B = 0.473 - 0.047 = 0.426$.

S - α - GC activity (U/g soil sample) = $0.444 \times \Delta A \div \Delta A_S \div W = 0.444 \times 0.149 \div 0.426 \div 0.02 = 7.7648$ U/g soil sample.

Related Products:

- BC0160/BC0165 Soil β -glucosidase (β - GC) Activity Assay Kit
- BC4040/BC4045 Soil Neutral Invertase(S-NI) Activity Assay Kit
- BC0240/BC0245 Soil Saccharase(S-SC) Activity Assay Kit