

## Soil $\beta$ -Xylosidase (S- $\beta$ -XYS) Activity Assay Kit

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

**Operation Equipment:** Spectrophotometer/ Microplate Reader

**Catalog Number:** BC4015

**Size:** 100T/48S

### Components:

**Reagent I:** Methylbenzene 2 mL $\times$ 1, Storage at 4°C (**self-provided reagent**).

**Reagent II:** Liquid 10 mL $\times$ 1. Storage at 4°C.

**Reagent III:** Powder $\times$ 2. Storage at -20°C. Add 5 mL of distilled water to each bottle before use.

The left reagent can be stored at -20°C for four weeks.

**Reagent IV:** Liquid 30 mL $\times$ 1. Storage at 4°C.

**Standard:** Liquid 1 mL $\times$ 1. Storage at 4°C. 5 mmol/L Phenol standard solution.

### Product Description:

Soil  $\beta$ -xylosidase (S- $\beta$ -XYS) exists in organisms such as plants, bacteria and fungi, which is a key enzyme that catalyzes the degradation of xylan hemicellulose. The product xylose can be used as a carbon source in microbial fermentation. In addition,  $\beta$ -XYS can also be used as a biological bleaching agent in the paper industry, which is more environment-friendly than traditional bleaching methods and has a widespread application value.

S- $\beta$ -XYS can catalyze the p-nitrophenyl beta-xylopyranoside to p-nitrophenol. The product has characteristic of absorption at 400 nm. In this kit, the S- $\beta$ -XYS activity is quantified by measuring the increase in the color development at 400 nm.

### Reagents and Equipment Required but Not Provided:

Spectrophotometer or microplate reader, water-bath, desk centrifuge, transferpettor, micro glass cuvette/96 well plate, analytical balance, mortar, 30-50 mesh sieve, **methylbenzene**, ice and distilled water.

### Procedure:

#### I. Preparation of samples

Fresh soil samples are naturally air-dried or oven-dried at 37°C, pass through a 30-50 mesh sieve.

#### II. Determination procedure:

1. Preheat microplate reader or spectrophotometer for 30 minutes, adjust the wavelength to 400 nm, spectrophotometer set zero with distilled water.
2. Dilution of standard solution: Take 20 $\mu$ L of 5 mmol/L p-nitrophenol solution before use, add 980 $\mu$ L of reagent II, mix well, and make a 100  $\mu$ mol/L standard solution for use, ready to use. (In the experiment, each tube needs 100 $\mu$ L, in order to reduce the experiment error, so prepare a large volume.)
3. Add reagents with the following list:

Reagent	Test tube (T)	Contrast Tube (C)	Standard tube (S)	Blank tube (B)
Air-dried soil (g)	0.02	0.02	-	-
Reagent I (μL)	5	5	-	-
The soil samples are all wetted by oscillating mixing, and store at 37°C for 15 minutes.				
Reagent II (μL)	100	100	-	-
Reagent III	80	-	-	-
Mix thoroughly and incubate the reaction for 1 hour at 50 °C water bath, then take the reaction solution in a boiling water bath for 5 minutes immediately (tightly close to prevent moisture loss), flowing water to cool.				
Reagent III (μL)	-	80	-	-
Mix thoroughly, centrifuge at 10000 rpm for 10 minutes at 25°C and take the supernatant.				
Supernatant (μL)	100	100	-	-
Standard solution (μL)	-	-	100	-
Distilled water (μL)	-	-	-	100
Reagent IV (μL)	200	200	200	200

Mix thoroughly and stand at room temperature for 2 minutes, centrifuge at 10000×g for 5 minutes. Take the supernatant and detect the absorbance of each tube at 400 nm, noted as  $A_T$ ,  $A_C$ ,  $A_S$  and  $A_B$ . Calculate  $\Delta A_T = A_T - A_C$ ,  $\Delta A_S = A_S - A_B$ . Each test tube should be provided with one contrast tube.

### III. S-NAG activity calculation:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the generation 1 μmol of p-nitrophenol every gram of soil sample in the reaction system per day.

$$S\text{-}\beta\text{-XYS (U/g soil sample)} = \Delta A_T \div (\Delta A_S \div C) \times V_{rv} \div W \div T = 0.444 \times \Delta A_T \div \Delta A_S \div W$$

C: Concentration of standard solution, 100 μmol/L;

$V_{rv}$ : Total volume in catalyze system,  $1.85 \times 10^{-4}$  L;

W: Soil sample weight, g;

T: React time, 1 hour = 1/24 day;

### Experimental Examples:

- Take two tubes of 0.02 g soil, which are the measuring tube and the control tube. Follow the measuring steps and mark them as  $A_t$  and  $A_c$ . Calculate  $\Delta A_t = A_t - A_c = 0.297 - 0.128 = 0.169$ ,  $\Delta A_s = A_s - A_b = 0.395 - 0.047 = 0.348$ , calculate the enzyme activity:

$$S\text{-NAG activity (U/g soil)} = 0.444 \times \Delta A_t \div \Delta A_s \div W = 0.444 \times 0.169 \div 0.348 \div 0.02 = 10.781 \text{ U/g soil.}$$

- Take two tubes of 0.02 g forest soil samples, which are the measuring tube and the control tube. Follow the measuring steps and mark them as  $A_t$  and  $A_c$ . Calculate  $\Delta A_t = A_t - A_c = 0.251 - 0.089 = 0.162$ ,  $\Delta A_s = A_s - A_b = 0.395 - 0.047 = 0.348$ , calculate enzyme activity:

$$S\text{-NAG activity (U/g soil)} = 0.444 \times \Delta A_t \div \Delta A_s \div W = 0.444 \times 0.162 \div 0.348 \div 0.02 = 10.344 \text{ U/g soil}$$

### Related Products:

BC3080/BC3085 Soil α-glucosidase(S-α-GC) Activity Assay Kit

BC0240/BC0245 Soil Saccharase(S-SC) Activity Assay Kit