

## **Soil amylase (S-AL) Activity Assay Kit**

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

**Operation Equipment:** Spectrophotometer

**Catalog Number:** BC1920

**Size:**50T/24S

### **Components:**

Reagent 1: 10 mL×1, storage at 4°C.

Reagent 2: Powder×1, storage at 4°C. Before use, add 5 mL of distilled water, place in normal temperature water and heat to boiling. During this period, keep shaking until the powder is dissolved.

Reagent 3: 65 mL×1, storage at 4°C.

Standard: powder×1, 10 mg of maltose, storage at 4°C. Add 1.38 mL of distilled water to prepare 20 μmol/mL standard solution.

### **Product Description:**

Amylase (EC 3.2.1.1) is a general term for a class of enzymes that catalyze the hydrolysis of starch. Soil amylase mainly comes from microorganisms, is an important enzyme preparation, widely used in food processing, food, brewing, fermentation, textile industry and pharmaceutical industry.

Amylase hydrolyzes starch to produce reducing sugar, which can react with 3,5-dinitrosalicylic acid to produce a red-brown substance. It has a characteristic absorption peak at 540 nm, and the color depth is proportional to the amount of reducing sugar within a certain range.

### **Reagents and Equipments Required but Not Provided:**

Spectrophotometer, adjustable transferpettor, balance, mortar/homogenizer, low temperature centrifuge, water-bath, 1 mL glass cuvette, sieve (30-50 mesh, or smaller), toluene, ice and distilled water.

### **Sample preparation:**

Fresh soil samples are naturally air-dried or oven dried at 37°C and passing 30-50 mesh sieve.

### **Procedure:**

1. Preheat spectrophotometer for 30 min, adjust the wavelength to 540 nm and set the counter to zero

with distilled water.

- Dilute the standard solution with distilled water to prepare 1, 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3  $\mu\text{mol/mL}$  standard solution.
- Add reagent to a 1.5 mL EP tube:

Reagent name	Test tube (At)	Control tube (Ac)	Standard tube (As)	Blank tube (Ab)
Sample (g)	0.1	0.1		
Toluene ( $\mu\text{L}$ )	20	20		
Distilled water ( $\mu\text{L}$ )		200		
Reagent 1 ( $\mu\text{L}$ )	200	200		
Reagent 2 ( $\mu\text{L}$ )	200			
Mix well and incubate at 37°C for 24 hours . Centrifuge at 12000 rpm for 10 min at room temperature and take the supernatant.				
Supernatant ( $\mu\text{L}$ )	300	300		
Distilled water ( $\mu\text{L}$ )				300
Standard solution ( $\mu\text{L}$ )			300	
Reagent 3 ( $\mu\text{L}$ )	700	700	700	700

Mix well and react in boiling water for 10 min. After cooling, the absorbance at the wavelength of 540 nm in 1 mL glass cuvette, and record them as At, Ac, As, and Ab, and calculate  $\Delta A = A_t - A_c$ ,  $\Delta A_s = A_s - A_b$ . The blank tube only needs to be tested 1-2 times.

### Calculation:

#### 1. Standard curve

According to concentration of standard solution and absorbance to create the standard curve, take standard solution as X-axis,  $\Delta A_s$  as Y-axis. Take  $\Delta A$  into the equation to obtain x ( $\mu\text{mol/mL}$ ).

#### 2. Calculate:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1  $\mu\text{mol}$  of reducing sugar in the reaction system per day every g soil.

$$\text{S-AL (U/g soil sample)} = x \times V_r \div T \div W = 0.42 \times x \div W$$

T: reaction time, 1 d;

V<sub>r</sub>: reaction volume, 0.42 mL;

W: soil weight, g;

**Note:**

1. When the  $\Delta A$  is greater than 1, it is recommended to further dilute the supernatant and measure.
2. If the determination of  $\Delta A$  is small, increase the volume of the supernatant of the reaction or increase the enzymatic reaction time appropriately, modify the formula when calculating the enzyme activity.
3. Please measure the absorbance within 30 min.

**Experimental example:**

Take 0.1g grass to 1.5ml EP tube, add 20  $\mu$  L toluene, 200  $\mu$  L reagent 1, 200  $\mu$  L reagent 2 as test tube ;  
Take 0.1g grass to 1.5ml EP tube, add 20  $\mu$  L toluene, 200  $\mu$  L reagent 1, 200  $\mu$  L distilled water as control tube, culture for 24h at 37°C . Centrifuge and take the supernatant, dilute 5 times, operate as the procedure,  $\Delta A_t = A_t - A_c = 0.624 - 0.109 = 0.515$ , standrad curve:  $y = 1.289x - 0.2826$ ,  $x = 0.6188$ , calculate enzyme activity by sample weight: S-AL (U/g weight) =  $0.42 \times x \div W \times 5(\text{dilute times}) = 0.42 \times 0.6188 \div 0.1 \times 5(\text{dilute times}) = 12.995$  U/g weight.

**References:**

[1] Kathiresan K, Manivannan S.  $\alpha$ -Amylase production by *Penicillium fellutanum* isolated from mangrove rhizosphere soil[J]. African journal of Biotechnology, 2006, 5(10).

[2] Ebregt A, Boldewijn J. Influence of heavy metals in spruce forest soil on amylase activity, CO<sub>2</sub> evolution from starch and soil respiration[J]. Plant and Soil, 1977, 47(1): 137-148.

**Related products:**

BC0120/BC0125 Soil Urease(UE) Activity Assay Kit

BC0100/BC0105 Soil Catalase(S-CAT) Activity Assay Kit