Soil amylase (S-AL) Activity Assay Kit

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

Operation Equipment: Spectrophotometer/ Microplate reader

Catalog Number: BC1925

Size:100T/48S

Components:

Reagent 1: 10 mL×1, storage at 4℃.

Reagent 2: Powder×1, storage at 4℃. 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. The left reagent can be stored at 4°C for four weeks.

Reagent 3: 20 mL×1, storage at 4℃.

Standard: powder×1, 10 mg of maltose, storage at 4℃. 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/Microplate reader, adjustable transferpettor, balance, mortar/homogenizer, low temperature centrifuge, water-bath, micro glass cuvette/96 well flat-bottom plate, 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℃ and passing 30-50 mesh sieve.

Procedure:

1. Preheat spectrophotometer/ microplate reader for 30 min, adjust the wavelength to 540 nm and set the counter to zero with distilled water.
2. Dilute the standard solution with distilled water to prepare 2, 1, 0.8, 0.6, 0.4 μmol/mL standard solution
3. 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)	0.05	0.05		
Toluene (μL)	10	10		

Distilled water (μL)		100		
Reagent 1 (μL)	100	100		
Reagent 2 (μL)	100			
Mix well and incubate at 37 ° C for 24 hours . Centrifuge at 12000rpm for 10min at room temperature and take the supernatant.				
Supernatant (μL)	60	60		
Distilled water (μL)				60
Standard solution (μL)			60	
Reagent 3（μL）	140	140	140	140

Mix well and react in boiling water for 10 min. After cooling, the absorbance at the wavelength of 540 nm in a micro glass cuvette/96 well flat-bottom plate, and record them as At, Ac, As, and Ab, and calculate ΔA = At-Ac, ΔAs = As-Ab. 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, ΔAs as Y-axis. Take ΔA into the equation to obtain x (μmol/mL).

2. Calculate:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 μmol of reducing sugar in the reaction system per day every g soil.

S-AL (U/g soil sample) = x×Vr÷T÷W=0.21×x÷W

T：reaction time, 1 d;

Vr: reaction volume, 0.21 mL;

W: soil weight, g;

Note:

1. When the ΔA is greater than 1.5, it is recommended to further dilute the supernatant and measure.

2.If the determination of ΔA 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. It is recommended that the cooling time after boiling in each experiment be the same ,please measure the absorbance within 30 min.

Experimental example:

Take 0.05g grass to 1.5ml EP tube, add 10μL toluene, 100μL reagent 1, 100μL reagent 2 as test tube; Take 0.05g grass to 1.5ml EP tube, add 10μL toluene, 100μL reagent 1, 100μL distilled water as control tube, culture for 24h at 37℃. Centrifuge and take the supernatant, dilute 3 times, operate as the procedure, ΔAt=At-Ac=0.948-0.12=0.828, standrad curve: y=0.7604x-0.158，x=1.297, calculate enzyme activity by sample weight: S-AL （ U/g weight)=0.21×x÷W×3(dilute

times)=0.21×1.297÷0.05×3(dilute times)=16.34 U/g weight.

References：

[1] Kathiresan K, Manivannan S. α-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 2 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