SunLong Biotech Co.,LTD
Tel: 0086-571-56623320 Fax:0086-571-56623318
E-mail:sales@sunlongbiotech.com
www.sunlongbiotech.com

Soil Laccase Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Cat No: AK0331

Size: 50T/24S

Components:

Reagent I: Liquid 60 mL×1, store at 4℃;

Reagent II: Powder×2, store at 4℃ and protect from light. Add 15 mL Reagent I when the solution will be used;

Reagent III: Liquid 3 mL×1, store at room temperature. If it has white matter precipitation, it can be dissolved in 37℃.

Product Description:

Soil laccase (SL) is a polyphenol oxidase containing copper. It belongs to the ceruloplasmic oxidase family. It is widely distributed in fungi and higher plants. At the same time, it is widely used in pulp biobleaching, environmental pollutant degradation, lignocellulose degradation and biological detection.

Laccase can decompose substrate ABTS to produce ABTS free radicals. Its absorption coefficient at 420nm is much higher than that of ABTS. Laccase activity can be calculated by measuring the increasing rate of ABTS radicals.

Required but Not Provided:

Spectrophotometer, balance, low temperature desk centrifuge, transferpettor, oscillating instrument, 1 mL glass cuvette, 30-50 mesh sieve and distilled water.

Protocol

I. Preparation:

Air dry the fresh soil sample and sieve it through 30-50 meshes.

II. Determination procedure:

1. Preheat spectrophotometer for 30 minutes, adjust wavelength to 420 nm, set the counter to zero with

distilled water.

2. Operation table:

Reagent (μL)	Test tube (A_T)	Contract tube (A_C)
Soil sample (g)	0.1	0.1
Reagent I	450	450
Reagent II	500	-
Place the reaction in the water bath at 37℃ for 10 min.		
Reagent III	50	50
Reagent II	-	500
Centrifuge at 4℃ 12000 g for 15 min. Take 200 μL supernatant and measure its absorbance at 420 nm. Record as A_T, A_C. $\Delta A=A_T-A_C$.		

III. Soil laccase (SL) Calculation:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the generation of 1 nmol ABTS free radical in the reaction system per minute every g soil.

SL activity(U/g weight)=$\Delta A\div(\varepsilon\times d)\times 10^9\times V_{RT}\div W\div T=2.78\times\Delta A\div W$

ε: ABTS free radical molar extinction coefficient, 36000 L/mol/cm;

d: Light path of cuvette, 1 cm;

V_{RT}: Total reaction volume, 0.001 L;

W: Sample weight, g;

10^9: Unit conversion factor, 1 mol=10^9 nmol;

T: Reaction time, 10 min;

Note:

1. The reagent shall be prepared before use and used as soon as possible. It shall be stored at 4℃ for one week. If it changes color, it cannot be used.

2. Do pre experiment before measurement. If the absorption value is high (A>1.5). Please reduce the

weight of the soil sample for further measurement. If the value is too small, the reaction time can be prolonged or the weight of soil sample can be increased.

3. If the supernatant is still turbid after centrifugation. It can be removed by centrifugation again.

Experimental example:

1. Take 2 pieces 0. 1g grass to 1.5ml EP tube, one is test tube and the other is contract tube, operate as the procedure, $\Delta A=A_T-A_C=0.698-0.334=0.364$, calculate content by sample weight: SL Activity （U/g weight)= 2.78×ΔA÷W=2.78×0.364÷0. 1=10. 12 U/g weight.
2. Take 2 pieces 0. 1g forest soil to 1.5ml EP tube, one is test tube and the other is contract tube, operate as the procedure, $\Delta A=A_T-A_C=1.43-0.778=0.652$, calculate content by sample weight: SL Activity （U/g weight)= 2.78×ΔA÷W=2.78×0.652÷0. 1=18. 13 U/g weight.

Related products：

AK0594/AK0593 Soil Polyphenoloxidase Activity Assay Kit

AK0592/AK0591 Soil Urease(UE) Activity Assay Kit

AK0574/AK0573 Soil Saccharase(S-SC) Activity Assay Kit