

## Soil Laccase 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:** AK0330

**Size:** 100T/48S

### Components:

Reagent I: Liquid 30 mL×1, store at 4°C;

Reagent II: Powder×2, store at 4°C and protect from light. Add 7.5 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°C .

### 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/microplate reader, balance, low temperature desk centrifuge, transferpettor, oscillating instrument, micro glass cuvette/96 well flat-bottom plate, 30 mesh sieve (or smaller) and distilled water.

### Protocol

#### I. Preparation:

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

#### II. Determination procedure:

1. Preheat spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 420 nm, set the

counter to zero with distilled water.

## 2. Operation table:

Reagent ( $\mu\text{L}$ )	Test tube ( $A_T$ )	Blank tube ( $A_B$ )
Soil sample (g)	0.03	0.03
Reagent I	135	135
Reagent II	150	-
Place the reaction in the water bath at 37°C for 10 min.		
Reagent III	15	15
Reagent II	-	150
Centrifuge at 4°C 12000 g for 15 min. Take 200 $\mu\text{L}$ 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:

### a. Micro glass cuvette

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.

$$\text{SL activity (U/g weight)} = \Delta A \div (\epsilon \times d) \times 10^9 \times V_{RT} \div W \div T = 0.833 \times \Delta A \div W$$

$\epsilon$ : ABTS free radical molar extinction coefficient, 36000 L/mol/cm;

d: Light path of cuvette, 1 cm;

$V_{RT}$ : Total reaction volume,  $3 \times 10^{-4}$  L;

W: Sample weight, g;

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

T: Reaction time, 10 min.

### b. 96 well flat-bottom plate

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.

$$\text{SL activity (U/g weight)} = \Delta A \div (\epsilon \times d) \times 10^9 \times V_{\text{RT}} \div W \div T = 1.39 \times \Delta A \div W$$

$\epsilon$ : ABTS free radical molar extinction coefficient, 36000 L/mol/cm;

d: Light path of cuvette, 0.6 cm;

$V_{\text{RT}}$ : Total reaction volume,  $3 \times 10^{-4}$  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°C 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 0.03g grass to 1.5ml EP tube, one is test tube and the other is contract tube, operate as the procedure,  $\Delta A = A_T - A_C = 0.421 - 0.23 = 0.191$ , calculate content by sample weight: SL Activity (U/g weight) =  $1.39 \times \Delta A \div W = 1.39 \times 0.191 \div 0.03 = 8.84$  U/g weight.
2. Take 2 pieces 0.03g forest soil to 1.5ml EP tube, one is test tube and the other is contract tube, operate as the procedure,  $\Delta A = A_T - A_C = 1.009 - 0.463 = 0.546$ , calculate content by sample weight: SL Activity (U/g weight) =  $1.39 \times \Delta A \div W = 1.39 \times 0.546 \div 0.1 = 25.30$  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