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CheKine™ Micro Laccase Activity Assay Kit

Cat #: KTB3029 Size: 48 T/48 S 96 T/96 S

	Micro Laccase Activity Assay Kit		
REF	**Cat #:** KTB3029	LOT	**Lot #:** Refer to product label
	Applicable sample: Plant Tissues, Fungus		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Laccase is a kind of copper-containing polyphenol oxidase, belonging to ceruloplasmin oxidase family, which is widely distributed in fungi and higher plants. Laccase has strong redox ability and is widely used in pulp biological bleaching, degradation of environmental pollutants and lignocellulose, and biological detection. CheKine™ Micro Laccase Activity Assay Kit can detect biological samples such as plant tissues, fungus. In this kit, laccase decomposes substrate ABTS to produce ABTS free radicals, and the absorption coefficient at 420 nm is much larger than that of substrate ABTS. The laccase activity can be calculated by measuring the increase rate of ABTS free radicals.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	60 mL	120 mL	4℃
Reagent Ⅰ	20 mL	40 mL	4℃
Reagent Ⅱ	Powder×1 vial	Powder×2 vials	4℃, protected from light

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 420 nm
- 96-well microplate or microglass cuvette, precision pipettes, disposable pipette tips,1.5 mL EP tube
- Incubator, ice maker, freezing centrifuge
- Deionized water
- Homogenizer or mortar (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.

Reagent I: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Working Reagent II: Prepared before use. Add 15 mL Reagent Ⅰ to each bottle, dissolve thoroughly. The remaining reagent can also be stored at 4℃ and protected from light for 1 week. The prepared Working Reagent Ⅱ should be colorless and transparent. If the reagent changes color, it cannot be used.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month.When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

1. Plant tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize or mortar on ice. Centrifuge at 12,000 g for 30 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Fungus: Collect 5×10^6 fungus into the centrifuge tube, wash fungus with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the fungus 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 12,000 g for 30 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 420 nm, visible spectrophotometer was returned to zero with deionized water.
2. Operation table (The following operations are operated in the 96-well microplate or microglass cuvette):

Reagent	Test Well（μL）	Blank Well（μL）
Sample	30	0
Deionized water	0	30
Working Reagent Ⅱ	170	170

3. Mix well, measure the absorbance value A_1 at 10 s at 420 nm immediately, Accurate incubation at 45℃ for 3 min, record the absorbance value A_2 at 190 s at 420 nm. The Blank Well is marked as A_{Blank}, and the Test Well is marked as A_{Test}. Finally calculate $\Delta A_{Blank}=A_{2Blank}-A_{1Blank}$, $\Delta A_{Test}=A_{2Test}-A_{1Test}$, $\Delta A=\Delta A_{Test}-\Delta A_{Blank}$.

Note: (1) The Blank Well only need to be done once or twice. The Blank Well is a detection hole for detecting the quality of each reagent component. Under normal circumstances, the OD value changes no more than 0.05. (2) In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA is less than 0.01, increase the sample quantity appropriately. If ΔA is larger than 1.0, the supernatant can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

A. 96-well plates calculation formula as below

(1) Calculated by protein concentration

Active unit definition: The amount of enzyme required to produce 1 nmol ABTS free radical per gram of soil per minute was defined as one unit of enzyme activity.

Laccase (U /mg prot)=$\Delta A\div(\varepsilon\times d)\times10^9\times V_{Total}\div(V_{Sample}\times Cpr\div V_{Sample\ total})\div T$**=123.46×ΔA÷Cpr**

(2) Calculated by fresh weight of samples

Active unit definition: The amount of enzyme required to produce 1 nmol ABTS free radical per gram of soil per minute was

defined as one unit of enzyme activity.

Laccase (U /g fresh weight)=ΔA÷(ε×d)×10^9×V_{Total}÷(V_{Sample}×W÷$V_{Sample\ total}$)÷T**=123.46×ΔA÷W**

(3) Calculated by the number of fungus

Active unit definition: The amount of enzyme required to produce 1 nmol ABTS free radical per gram of soil per minute was defined as one unit of enzyme activity.

Laccase (U /10^4 fungus)=ΔA÷(ε×d)×10^9×V_{Total}÷(V_{Sample}×N÷$V_{Sample\ total}$)÷T**=123.46×ΔA÷N**

ε: ABTS free radical molar extinction coefficien, 3.6×10^4 L/mol/cm; d: 96-well plate diameter, 0.5 cm; V_{Total}: Total volume of reaction system, 0.2 mL=2×10^{-4} L; V_{Sample}: Added the sample volume, 0.03 mL; $V_{Sample\ total}$: Added the Extraction Buffer volume, 1 mL; Cpr: sample protein concentration, mg/mL; W: Sample weight, g; N: Fungus counts in tens of thousands; T: reaction time, 3 min; 10^9: Unit conversion coefficient, 1 mol=1×10^9 nmol.

B. Microglass cuvette calculation formula

The optical diameter d: 0.5 cm in the above calculation formula can be adjusted to d: 1 cm for calculation.

Typical Data

The following data are for reference only. And the experimenters need to test the samples according to their own experiments.

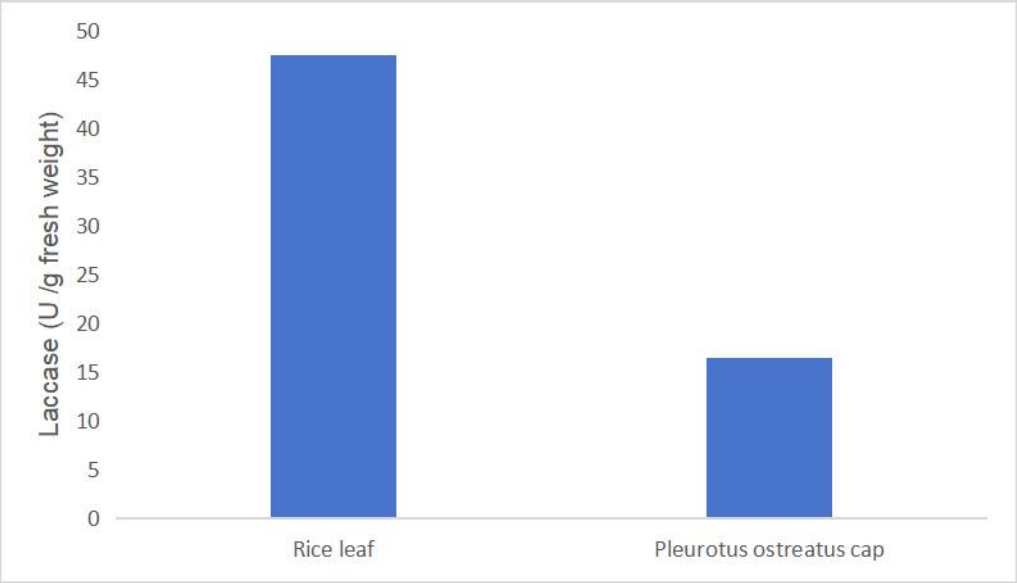

Figure 1. Determination laccase activity in rice leaf and pleurotus ostreatus cap by this assay kit.

Recommended Products

Catalog No.	Product Name
KTB1150	CheKine™ Micro Peroxidase (POD) Activity Assay Kit
KTB1030	CheKine™ Micro Superoxide Dismutases (SOD) Activity Assay Kit
KTB1040	CheKine™ Micro Catalase (CAT) Content Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.