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Laccase Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Cat No: AK0319

Size: 50T/48S

Components:

Extract reagent: Liquid 60 mL×1, store at 4℃;

Reagent I: Liquid 50 mL×1, store at 4℃;

Reagent II: Powder×2, store at 4℃ and protect from light.

Product Description:

Laccase (EC1. 10.3.2) is a polyphenol oxidase containing copper. It belongs to the ceruloplasmic oxidase family. Laccase is a kind of environmental protection enzyme which exists in mushroom, fungus and plant. Its unique catalytic properties are widely used in 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, mortar/ homogenizer, water bath, ice and distilled water.

Protocol

I. Preparation:

(1) Tissue: according to the ratio of mass (g): extraction volume (mL): 1:5- 10 to add the extract reagent. It is suggested that add 1 mL of extract to 0.1 g of tissue. Homogenate on ice. Centrifuge at 10000 g 4℃ for 10 min. Take the supernatant on ice for test.

(2) Cells: according to the number of the cells (10^4): the volume of the extract (mL) is 500~1000:1. It is suggested that add 1 mL of extraction reagent to 500 million of cells. Breaking cells by ultrasonic wave in

ice bath (power 300W, ultrasonic 3s, interval 7s, total time 3 min). Centrifuge at 10000 g 4℃ for 10 min. Take the supernatant on ice for test.

(3) Culture medium: direct detection.

II. Determination procedure:

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

2. Adjust the temperature of the water bath to 45℃ .

3. Working solution: a bottle of Reagent II is dissolved with 25 mL of Reagent I. Use now and match now.

4. Operation table: add the following reagents to the 1 mL glass cuvette respectively:

Reagent (μL)	Test tube (A_T)	Blank tube (A_B)
Sample	150	
Distilled water	-	150
Working solution	850	850
Add the above reagents into the 1 mL glass cuvette respectively. Mix thoroughly. Measure the absorbance value A1 at 420 nm for 10s. Quickly put it into a 45℃ water bath for 3 min. Take out and dry it, then measure the absorbance value A2 of 190s. $\Delta A_T=A2_T-A1_T$. $\Delta A_B=A2_B-A1_B$. $\Delta A=A_T-A_B$. Blank tube only needs to test once or twice.		

III. Laccase Calculation:

(1) Protein concentration

Unit definition: One unit of enzyme is defined as the amount of enzyme oxidation of 1 nmol ABTS in the reaction system per minute every mg protein.

Laccase activity (U/mg prot)=$\Delta A\div(\varepsilon\times d)\times V_{RT}\times 10^9\div(V_S\times Cpr)\div T=61.7\times\Delta A\div Cpr$

(2) Sample weight

Unit definition: One unit of enzyme is defined as the amount of enzyme oxidation of 1 nmol ABTS in the reaction system per minute every g sample.

Laccase activity (U/g weight)=$\Delta A\div(\varepsilon\times d)\times V_{RT}\times 10^9\div(V_S\times W\div V_{ST})\div T=61.7\times\Delta A\div W$

(3) Cells

Unit definition: One unit of enzyme is defined as the amount of enzyme oxidation of 1 nmol ABTS in the

reaction system per minute every 10^4 cells.

Laccase activity (U/10^4 cells)=ΔA÷(ε×d)×V_{RT}×10^9÷(V_S×500÷V_{ST})÷T=0. 123×ΔA

(4) Liquid volume

Unit definition: One unit of enzyme is defined as the amount of enzyme oxidation of 1 nmol ABTS in the reaction system per minute every mL liquid.

Laccase activity (U/mL)=ΔA÷(ε×d)×V_{RT}×10^9÷V_S÷T=61.7×ΔA

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;

V_S: Sample volume, 0.15 mL;

V_{ST}: Extract volume, 1 mL;

Cpr: Sample protein concentration, mg/mL;

W: Sample weight, g;

T: Reaction time, 3 min;

Note:

1. Prepare the working solution when it will be used. And use it as soon as possible. Keep it at 4℃ for one week. If it changes color, it cannot be used.

2. Carry out pre experiment before determination. If the absorbance value is high, please dilute the sample with the extraction solution for appropriate re determination. And multiply the dilution ratio in the calculation formula.

3. The blank tube is a test tube for testing the quality of each reagent component. Under normal conditions, the OD value does not change more than 0.05.

Experimental example:

1. Take 0. 1g mushroom to 1ml extract solution, supernatant is ready for test, operate as the procedure, $\Delta A_T=A2_T-A1_T=0.657-0.084=0.573$,$\Delta A_B=A2_B-A1_B=0.087-0.058=0.029$,$\Delta A=A_T-A_B=0.573-0.029=0.544$, calculate content by sample weight: Laccase Activity （U/g weight)= 61.7× Δ A÷ W=61.7×0.544÷0. 1=335.648 U/g weight.

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