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: BC1635

Size: 100T/96S

Components:

Extract reagent: Liquid 110 mL×1, store at 4℃;

Reagent II: Liquid 20 mL×1, store at 4℃;

Reagent III: 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/microplate reader, balance, low temperature desk centrifuge, transferpettor, oscillating instrument, micro glass cuvette/96 well flat-bottom plate, 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 extract 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/microplate reader for 30 minutes, 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 10 mL of Reagent I. Use now and match now.

4. Operation table: add the following reagents to the micro glass cuvette/96 well flat-bottom plate respectively:

Reagent (μL)	Test tube (A_T)	Blank tube (A_B)
Sample	30	
Distilled water	-	30
Working solution	170	170
Add the above reagents into the micro glass cuvette/96 well flat-bottom plate respectively. Mix thoroughly. Measure the absorbance value A1 at 420 nm for 10s. Quickly put it into a 45℃ water bath for 3 min. If the microplate reader has temperature control function, the temperature can be adjusted to 45℃. 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:

a. Micro glass cuvette

(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) $=\Delta A \div(\varepsilon \times d) \times V_{RT} \times 10^9 \div(V_S \times 500 \div V_{ST}) \div T=0.123 \times \Delta A$

(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) $=\Delta A \div(\varepsilon \times d) \times V_{RT} \times 10^9 \div V_S \div T=61.7 \times \Delta A$

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

d: Light path of cuvette, 1 cm;

V_{RT}: Total reaction volume, 2×10^{-4} L;

V_S: Sample volume, 0.03 mL;

V_{ST}: Extract volume, 1 mL;

Cpr: Sample protein concentration, mg/mL;

W: Sample weight, g;

T: Reaction time, 3 min;

b. 96 well flat-bottom plate

In the above calculation formula, d-1cm is replaced by d-0.6cm (96 well flat-bottom plate optical diameter) for calculation.

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. 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, $\Delta A_T=A2_T-A1_T=0.8963-0.1004=0.7959$,$\Delta A_B=A2_B-A1_B=0.0729-0.0542=0.0187$,$\Delta A=A_T-A_B=0.7959-0.0187=0.07772$, calculate content by sample weight: Laccase Activity（U/g weight)= 61.7×ΔA÷W=61.7×0.7772÷0.1=479.53U/g weight.

Related products：

BC0200/BC0205 Catalase(CAT) Activity Assay Kit

BC0090/BC0095 Peroxidase(POD) Activity Assay Kit