Lignin Peroxidase (Lip) activity Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer

Cat No: BC1610

Size: 50T/48S

Components:

Reagent I : Liquid 85mL×1. Storage at 2-8℃.

Reagent II : Liquid 11 mL×1. Storage at 2-8℃.

Reagent III : Liquid 6 mL×1. Storage at 2-8℃.

Product Description:

Lignin peroxidase (EC1.11.1.14) （Lip）is a peroxidase containing heme, belonging to the lignin degrading enzyme system, used in lignin biodegradation, paper industry, textile industry, aromatic compound conversion and Degradation and environmental pollution control have great application potential.

Lignin peroxidase oxidizes veratrol to generate veratraldehyde, which has a characteristic absorption peak at 310nm.

Reagents and Equipment Required but Not Provided:

Spectrophotometer, Refrigerated Centrifuge, Water-bath/Constant Temperature Incubator, Adjustable Pipette, Cell Ultrasonic Crusher, Mortar/Homogenizer, Ice, Distilled water

Sample Preparation：

1. Tissue

The proportion of tissue mass (g): reagent I volume (ml) of 1:5~10 (it is recommended to weigh about 0.1 g of tissue and add 1 mL of reagent I), ice bath homogenate, centrifuge at 10000×g for 10 minutes at 4℃ to remove insoluble materials. Take the supernatant and place it on ice for testing.

2. Bacteria or cells

The ratio of bacteria/cell amount (10^4): reagent I volume(mL) is 500~1000: 1 (it is suggested to take about 5 million bacteria/cell and add 1 mL of reagent I). Bacteria and cell is split by ultrasonic (placed on ice, 300W, work time 3s, interval 7s, for 3min). Centrifuge at 10000×g for 10 minutes at 4℃ to remove insoluble materials. Take the supernatant and place it on ice for testing.

3. Culture medium or other liquids: direct detection. If the solution is turbid, take the supernatant after centrifugation for determination

II. Determination procedure:

1. Preheat spectrophotometer for 30 minutes, adjust wavelength to 310 nm, set zero with distilled water.

2. Before determination, take out part of reagent I, reagent II, reagent III according to the experimental dosage and preheat them at 37 ℃ for 10min. If there are too many samples to be measured at

one time, prepare reagent I, II and III into working solution in the ratio of 6:2:1 according to the amount used, and then preheat them. During the measurement, it shall be determined according to 100 μL sample + 900 μLworking solution into 1ml glass cuvette for determination.

Reagent name（μL）	Test tube (T)
Reagent I（μL）	600
Reagent II（μL）	200
Sample（μL）	100
Reagent III（μL）	100
Mix thoroughly, adjust to zero in a 1mL quartz cuvette with distilled water, detect the absorbance at 310 nm at the time of 15 seconds record as A1, put the mixed solution in a 37 ℃ water bath / constant temperature incubator for cultivation for 5min, take out and measure the absorbance value A2 at 5min15s, and calculate Δ A = A2-A1, pay attention to ensure the accuracy of measurement time.	

III. Calculation:

1. Protein concentration

Unit definition: 37℃, pH 4.5, one unit of enzyme activity is defined as the amount of oxidize 1nmol veratrol per milligram of tissue protein per minute.

Lip（U/mg prot）=ΔA÷（ε×d）×Vr ×10^9÷（Vs×Cpr）÷T= 215.05×ΔA÷Cpr

2. Sample weight:

Unit definition: 37℃, pH 4.5, one unit of enzyme activity is defined as the amount of oxidize 1nmol veratrol per gram of tissue per minute.

Lip（U/g weight）=ΔA÷（ε×d）×Vr×10^9÷（Vs×W÷Ve）÷T= 215.05×ΔA÷W

3. Bacteria or cells

Unit definition: 37℃, pH 4.5, One unit of enzyme activity is defined as the amount of oxidize 1nmol veratrol per 10^4 of cell per minute.

Lip（U/10^4 cell）=ΔA÷（ε×d）×Vr×10^9÷（Vs×number of cells÷Ve）÷T= 215.05×ΔA÷number of cells

4. Liquid volume

Unit definition: 37℃, pH 4.5, One unit of enzyme activity is defined as the amount of oxidize 1nmol veratrol per milliliter of culture medium per minute.

Lip（U/mL）=ΔA÷（ε×d）×Vr×10^9÷Vs÷T= 215.05×ΔA

ε：Veratraldehyde molar extinction coefficient：9300L/mol/cm；d：Cuvette light path，1cm；Vr：Total reaction volume，0.001L；Vs: Sample volume，0.1mL；Ve：Extract，1mL；Cpr：Protein concentration，mg/mL；W：Sample weight g；T：Reaction time, 5min; 10^9：Unit conversion factor，1mol=10^9nmol。

Experimental example:

Take 0.09g Pleurotus eryngii and add 1 mL Reagent I for ice bath homogenization, then centrifugation at 4℃ and 10000g for 10min, take the supernatant, then operate according to the

determination steps, measure with 96 well plate and calculate ΔA =A2-A1 =0.55-0.228=0.322, calculate the enzyme activity according to the sample mass:

Lip activity （U/g weight）=ΔA×Vr÷(ε×d)×109÷Vs÷T=672.95U/g weight。

Recent Product Citations：

Konadu K T, Harrison S, Osseo-Asare K, et al. Transformation of the carbonaceous matter in double refractory gold ore by crude lignin peroxidase released from the white-rot fungus[J]. International Biodeterioration & Biodegradation, 2019, 143(1996):104735.

Ahmed A A Q, Mckay T J M. Potential of Bacillus sp. LG7 as a Promising Source of Ligninolytic Enzymes for Industrial and Biotechnological Applications[J]. Proceedings of the National Academy of ences India, 2017.

Related Products：

BC1630/BC1635 Laccase Assay Kit

BC1620/BC1625 Manganese peroxidase（Mnp）activity Assay Kit

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