Lignin Peroxidase (Lip) activity Assay Kit

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

Operation Equipment: UV spectrophotometer / Microplate Reader

Cat No: BC1615

Size: 100T/96S

Components:

Reagent I : Liquid 115 mL×1. Storage at 2-8℃.

Reagent II : Liquid 5 mL×1. Storage at 2-8℃.

Reagent III : Liquid 3 mL×1. Storage at 2-8℃.

Product Description:

Lignin peroxidase (EC1.11.1.14) 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/Microplate Reader, Refrigerated Centrifuge, Water-bath/Constant Temperature Incubator, Adjustable Pipette, Cell Ultrasonic Crusher, Mortar/Homogenizer, Micro Quartz Cuvette/96- Well Plate, 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 3 min). 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/Microplate Reader for 30 minutes, adjust wavelength to 310 nm, the spectrophotometer needs to be zeroed 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 20μL sample+180μLworking solution into micro quartz cuvette/96 well UV flat-bottom plate,for determination.

Reagent Name（μL）	Test Tube (T)
Reagent I（μL）	120
Reagent II（μL）	40
Sample（μL）	20
Reagent III（μL）	20
Mix thoroughly, adjust to zero in a 1mL quartz cuvette with distilled water, detect the absorbance at 360 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 ΔA = A2-A1, pay attention to ensure the accuracy of measurement time.	

III. Calculation:

A. micro quartz cuvette

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）=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，2×10^{-4}L；Vs: Sample volume，0.02mL；Ve：Extract，1mL；Cpr：Protein concentration，mg/mL；W：Sample weight g；T：reaction time 5min.

B:96-well plate:

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= 358.42×Δ A÷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= 358.42×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= 358.42×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= 358.42×ΔA

ε：Veratraldehyde molar extinction coefficient：9300L/mol/cm；d：Cuvette light path，0.6cm；Vr：Total reaction volume，2×10^{-4}mL；Vs: Sample volume，0.02mL；VI：reagent I，1mL；Cpr：Protein concentration，mg/mL；W：Sample weight, g；T：reaction time, 5min.

Experimental example:

Take 0.1125g 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 =0.5322-0.2294=0.3028, calculate the enzyme activity according to the sample mass: Lip activity （U/g weight）=ΔA×Vr÷(ε×d)×10^9÷Vs÷T=142.09U/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.

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Related Products：

BC1630/BC1635	Laccase Assay Kit
BC1620/BC1625	Manganese peroxidase（Mnp）activity Assay Kit

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