Manganese peroxidase（Mnp）activity Assay Kit

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

Operation Equipment: Spectrophotometer / Microplate Reader

Cat No: BC1625

Size: 100T/96S

Components:

Reagent I : Liquid 120mL×1. Storage at 2-8℃.

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

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

Reagent IV : Liquid 200μL×1. Storage at 2-8℃. Before use, according to the required amount of reagent IV（μL) : distilled water（μL) = 1:49, the reagent should be prepared before use.

Product Description:

Manganese peroxidase (EC1.11.1.13) is a microbial lignin decomposing enzyme widely existing in bacteria and fungi. It plays a key role in the microbial lignin decomposing system. It can effectively degrade lignin and refractory compounds in wastewater and soil. It is widely used in industrial fields such as biological pulping, biological bleaching and biodegradation of pollutants.

Manganese peroxidase oxidizes guaiacol to tetra-o-methoxyl phenol in the presence of Mn^{2+}, with a characteristic absorption peak at 465 nm.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/Microplate Reader, Micro quartz cuvette/96 well plate, Low temperature centrifuge, Water bath/Constant temperature incubatorbalance, Pipette, Cell Sonicator, Mortar/ homogenizer, Ice and distilled water.

I. Sample Preparation：

1. Tissue

The proportion of tissue mass (g): Reagent I (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.

II. Determination procedure:

1. Preheat the spectrophotometer for more than 30 minutes, adjust the wavelength to 465nm, and adjust to

zero with distilled water.

2. Place reagent I, II, III, and IV at 37°C (mammals) or 25°C (other animals) for more than 10 minutes before measurement. If there are many samples for one-time measurement, you can mix reagents I, II, III, and IV according to the proportions (5:1:2:1). Place them at 37°C (mammals) or 25°C (other species) for more than 10 minutes, and add 20μL of sample to the test Measured with 180μL of mixed solution.
3. Sample measurement table

Reagent Name （μL）	Test Tube (T)
Sample （μL）	20
Reagent I （μL）	100
Reagent II （μL）	20
Reagent III （μL）	40
Reagent IV （μL）	20

Add the above reagents in order in a micro quartz cuvette/96 well plate, mix and time immediately, record the absorbance value A1 at 30s at 465nm and the absorbance value A2 after 10min30s. Calculate ΔA=A2-A1.

II. Calculation:

A. Micro quartz cuvette

1. Protein concentration

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

Mnp（U/mg prot）=[ΔA×Vr÷（ε×d）×10^9]÷（Vs×Cpr）÷T= 82.64×ΔA÷Cpr

2. Sample weight:

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

Mnp（U/g weight）=[ΔA×Vr÷（ε×d）×10^9]÷（Vs×W÷VI）÷T= 82.64×ΔA÷W

3. Bacteria or cells

Unit definition: When pH=4.5，one unit of enzyme activity is defined as the amount of oxidize 1nmol guaiacol per 10^4 of cell/bacteria per minute.

Mnp（U/10^4 cell）=[ΔA×Vr÷（ε×d）×10^9]÷（Vs×number of cells÷VI）÷T=82.64×ΔA÷number of cells

4. Liquid volume

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

Mnp（U/mL）=[ΔA×Vr÷（ε×d）×10^9]÷Vs÷T= 82.64×ΔA

ε：Guaiacol molar extinction coefficient：12100L/mol/cm；d：Cuvette light path，1cm；Vr：Total reaction volume，0.0002L；Vs：Sample volume，0.02mL；VI：Reagent I，1mL；Cpr：Protein concentration，mg/mL；W：Sample weight，g；T：Reaction time，10min；10^9：Unit conversion factor，1mol=10^9nmol.

B. **96-well plate:**

1. Protein concentration

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

Mnp（U/mg prot）=[ΔA×Vr÷（ε×d）×10^9]÷（Vs×Cpr）÷T= 137.74×ΔA÷Cpr

2. Sample weight:

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

Mnp（U/g weight）=[ΔA×Vr÷（ε×d）×10^9]÷（Vs×W÷VI）÷T= 137.74×ΔA÷W

3. Bacteria or cells

Unit definition: When pH=4.5，one unit of enzyme activity is defined as the amount of oxidize 1nmol guaiacol per 10^4 of cell/bacteria per minute.

Mnp（U/10^4 cell）=[ΔA×Vr÷（ε×d）×10^9]÷（Vs×number of cells÷VI）÷T=137.74×ΔA÷number of cells

4. Liquid volume

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

Mnp（U/mL）=[ΔA×Vr÷（ε×d）×10^9]÷V ÷T= 137.74×ΔA

ε：Guaiacol molar extinction coefficient：12100L/mol/cm；d：96 well plate light path，0.6cm；Vr：Total reaction volume, 2×10^{-4}L；Vs：Sample volume，0.02mL；VI：Reagent I，1mL；Cpr：Protein concentration，mg/mL；W：Sample weight，g；T：Reaction time，10min, 10^9：Unit conversion factor， 1mol=10^9nmol。

Note:

When A1 is more than 1.5, it is recommended to dilute the sample with reagent I for determination; If ΔA is too small, the sample size can be appropriately increased and the determination can be carried out again. Pay attention to modifying the calculation formula synchronously.

Experimental example:

Take 0.1002g 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 =0.064-0.055=0.009, calculate the enzyme activity according to the sample mass: Mnp activity （U/g weight）=ΔA×Vr÷(ε×d)×10^9÷Vs÷T=12.37 U/g weight。

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Recent Product Citations:

[1] Chowdhary P, Shukla G, Raj G, et al. Microbial manganese peroxidase: a ligninolytic enzyme
and its ample opportunities in research[J]. SN Applied Sciences, 2019, 1(1):45.

[2] Rogalski J, Lundell T, Leonowicz A, et al. Production of laccase, lignin peroxidase and manganese-dependent peroxidase by various strains of Trametes versicolor depending on culture conditions[J]. Polish Society of Microbiologists, 1991, 40(3-4):221-234.

Related Products:

BC1630/BC1635	Laccase Assay Kit
BC0200/BC0205	Catalase (CAT) Activity Assay Kit
BC0090/BC0095	Peroxidase (POD) Activity Assay Kit
BC2650/BC2655	Filter paper enzyme (FPA) Activity Assay Kit

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