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Manganese peroxidase（Mnp）activity Assay Kit

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

Operation Equipment: Spectrophotometer / Microplate Reader

Cat No: AK0800-100T-96S

Size: 100T/96S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle.

Reagent name	Size	Preservation Condition
Reagent I	Liquid 120 mL×1	2-8℃
Reagent II	Liquid 3 mL×1	2-8℃
Reagent III	Liquid 5 mL×1	2-8℃
Reagent IV	Liquid 200 μL×1	2-8℃

Solution Preparation:

1. Reagent II: Freezing at-20 ℃ is prohibited. (freezing on ice or close to the inner wall of the refrigerator at 2-8 ℃ may cause reagent freezing.)

2. Reagent IV: Before use, according to the sample size , the ratio of reagent IV(μL) : distilled water(μL) = 1:49, the reagent should be prepared before use.

Product Description:

Manganese peroxidase (Mnp, 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 glass cuvette/96 well plate, low temperature centrifuge, water bath/constant temperature incubator, balance, adjustable pipette, mortar/homogenizer/cell ultrasonic crusher, ice and distilled water.

Operation procedure:

I. Sample preparation(The sample size to be tested can be adjusted appropriately, and the specific proportion can be referred to the literature.)

1. Tissue sample: Add extract solution according to the ratio of tissue mass (g) : reagent I volume (mL) = 1:5 ~10 (it is recommended to weigh 0.1g sample and add 1.0mL reagent I volume), after ice bath

homogenization, centrifuge at 4℃, 10000g for 10min, take supernatant and placed on the ice for test.

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

3. Liquid sample: Detect sample directly. (If the solution is turbid, centrifuge to take the supernatant and then measure)

II. Determination procedure

1. Preheat spectrophotometer/microplate reader for more than 30 min, adjust the wavelength to 465 nm and set spectrophotometer zero with distilled water.
2. Before the determination, some reagents I, II, III and IV were taken out according to the experimental dosage and preheated at 37 ℃ (mammal) or 25 ℃ (other animals) for more than 10 min. If there are too many samples to be determined at one time, the reagents I, II, III and IV can be prepared into a working solution at a ratio of 5 : 1 : 2 : 1 according to the amount of use, and then preheated. When the determination is carried out, a micro glass cuvette / 96 well plate is added according to 20 μL sample + 180 μL working solution.
3. Operation table(Add the reagents in order in a micro glass cuvette/96 well plate)

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

Mix thoroughly, detect the absorbance value at 465 nm at the time of 30s record as A1, put the mixed solution in a 37 ℃ (mammal) or 25 ℃ (other animals) water bath/constant temperature incubator for 10min, take out and detect the absorbance value A2 at 10min30s, calculate ΔA = A2-A1.

III. Calculation:

A. Micro quartz cuvette

1. Calculate by sample 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 activity (U/mg prot)= $\Delta A \times V_T \div (\varepsilon \times d) \times 10^9 \div (V_S \times Cpr) \div T = 82.64 \times \Delta A \div Cpr$

2. Calculate by sample mass

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 activity (U /g mass)= $\Delta A \times V_T \div (\varepsilon \times d) \times 10^9 \div (V_S \times W \div V_E) \div T = 82.64 \times \Delta A \div W$

3. Calculate by the number of 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 activity (U/10^4 cell)= $\Delta A \times V_T \div (\varepsilon \times d) \times 10^9 \div (V_S \times N \div V_E) \div T = 82.64 \times \Delta A \div N$

4. Calculate by liquid volum

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 activity (U/mL)= $\Delta A \times V_T \div (\varepsilon \times d) \times 10^9 \div V_S \div T = 82.64 \times \Delta A$

ε: Guaiacol molar extinction coefficient, 12100 L/mol/cm; d: Micro glass cuvette light path, 1 cm; V_T: Total reaction volume, 2×10^{-4} L; V_S Sample volume, 0.02mL; V_E: Reagent I volume, 1mL; 10^9: Reduction coefficient, 1mol=10^9 nmol; Cpr: Sample protein concentration, mg/mL; N: The number of bacteria or cells, count by 10^4; W: Sample mass g; T: Reaction time, 10 min.

B. 96 well plate:

1. Calculate by sample 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 activity (U/mg prot)= $\Delta A \times V_T \div (\varepsilon \times d) \times 10^9 \div (V_S \times Cpr) \div T = 137.74 \times \Delta A \div Cpr$

2. Calculate by sample mass

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 activity (U /g mass)= $\Delta A \times V_T \div (\varepsilon \times d) \times 10^9 \div (V_S \times W \div V_E) \div T = 137.74 \times \Delta A \div W$

3. Calculate by the number of 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 activity (U/10^4 cell)= $\Delta A \times V_T \div (\varepsilon \times d) \times 10^9 \div (V_S \times N \div V_E) \div T = 137.74 \times \Delta A \div N$

4. Calculate by liquid volum

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 activity (U/mL)= $\Delta A \times V_T \div (\varepsilon \times d) \times 10^9 \div V_S \div T = 137.74 \times \Delta A$

ε: Guaiacol molar extinction coefficient, 12100L/mol/cm; d: 96 well plate light path, 0.6 cm; V_T: Total reaction volume, 2×10^{-4}L; V_S Sample volume, 0.02mL; V_E: Reagent I volume, 1mL; 10^9: Reduction coefficient, 1mol=10^9nmol; Cpr: Sample protein concentration, mg/mL; N: The number of bacteria or

cells, count by 10^4; W: Sample mass g; T: Reaction time, 10min.

Note:

When A1>1.5 or ΔA>0.6 (microplate reader ΔA>0.4), 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, A1=0.055, A2=0.064, calculate ΔA =A2-A1 =0.009, calculate the enzyme activity according to the sample mass:

Mnp activity (U/g mass)=$\Delta A \times V_T \div (\varepsilon \times d) \times 10^9 \div Vs \div T$=12.37 U/g mass