Polyphenol Oxidase (PPO) Activity Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer/ Microplate reader

Catalog Number: BC0195

Size: 100T/48S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation Condition
Extract solution	Liquid 55 mL×1	2-8℃
Powder I	Powder×1	2-8℃
Reagent I	Liquid 22 mL×1	2-8℃
Reagent II	Liquid 6 mL×1	2-8℃

Solution Preparation:

1. Extract solution: Add Powder I to Extract solution before use. The solution is a suspension. Shake it before use.

Product Description:

Polyphenol oxidase (PPO) is mainly found in animals, plants, microorganisms and culture cells. PPO is a copper-contained oxidase that oxidizes monophenols and diphenols to produce quinones. It is closely related to fruit and vegetable processing, tea quality and tissue culture.

PPO can catalyze o-dihydroxybenzene to produce quinones which has absorbance at 410 nm.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, refrigerated centrifuge, water bath, transferpettor, micro glass cuvette/96 well flat-bottom plate, mortar/homogenizer/cell ultrasonic crusher, ice and distilled water.

Protocol:

I. Sample Preparation.

1. **Bacteria or cells:** Collect bacteria or cells to centrifuge tube, and discard supernatant after centrifuging. Add 1 mL of Extract solution to 5 million of bacteria or cells and use ultrasonic breaking bacteria or cells. (place on ice, ultrasonic power 200W, working time 3s, interval 10s, repeat for 30 times). Centrifuge at 8000 ×g for 10 minutes at 4℃ to remove insoluble materials and take the supernatant on ice for testing.
2. **Tissue:** Add 1 mL of Extract solution to 0.1 g of tissue, and homogenate on ice. Centrifuge at 8000 ×g for 10 minutes at 4℃ to remove insoluble materials and take the supernatant on ice for testing.
3. **Serum (plasma) 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 30 minutes, adjust wavelength to 410 nm, set spectrophotometer counter to zero with distilled water.
2. Add reagents with the following list:

Reagent (μL)	Test tube (T)	Contrast tube (C)
Reagent I	200	200
Reagent II	50	50
Sample	50	-
Boiled sample	-	50
Incubate at 37°C (mammals) or 25°C (other species) water bath for 10 minutes. Heat in boiled water for 10 minutes (Wrap the sealing film to prevent bursting). Mix thoroughly, centrifuge at 5000 ×g for 10 minutes at room temperature, take the supernatant. Take 200μL of supernatant to micro glass cuvette or 96 well flat-bottom plate. Detect the absorbance of test tube and contrast tube at 410 nm, noted as A_T, A_C. $\Delta A=A_T-A_C$.		

Note: Every test tube need set a contrast tube. Different samples of crude enzyme solution can be added to different contrast tubes, and then heat in boiled water for 5 minutes.

III. Calculation.

A. Micro glass cuvette

1. Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the absorbance of 0.01 change at 410 nm in the reaction system per minute every milligram protein

PPO activity (U/mg prot) = $\Delta A \div 0.01 \times V_{RT} \div (Cpr \times V_S) \div T = 60 \times \Delta A \div Cpr$

2. Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the absorbance of 0.01 change at 410 nm in the reaction system per minute every gram tissue.

PPO activity (U/g weight) = $\Delta A \div 0.01 \times V_{RT} \div (W \div V_{ST} \times V_S) \div T = 60 \times \Delta A \div W$

3. Cells or bacteria:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the absorbance of 0.01 change at 410 nm in the reaction system per minute every 10^4 of cells or bacteria.

PPO activity (U/10^4 cell) = $\Delta A \div 0.01 \times V_{RT} \div (500 \div V_{ST} \times V_S) \div T = 0.12 \times \Delta A$

B. 96 well flat-bottom plate

1. Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the absorbance of 0.005 change at 410 nm in the reaction system per minute every milligram protein.

PPO activity (U/mg prot) = $\Delta A \div 0.005 \times V_{RT} \div (Cpr \times V_S) \div T = 120 \times \Delta A \div Cpr$

2. Sample weight:

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Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the absorbance of 0.005 change at 410 nm in the reaction system per minute every gram tissue.

PPO activity (U/g weight) $=\Delta A\div 0.005\times V_{RT}\div(W\div V_{ST}\times V_{S})\div T=120\times\Delta A\div W$

3. Cells or bacteria:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the absorbance of 0.005 change at 410 nm in the reaction system per minute every 10^4 of cells or bacteria.

PPO activity (U/10^4 cell) $=\Delta A\div 0.005\times V_{RT}\div(500\div V_{ST}\times V_{S})\div T=0.24\times\Delta A$

V_{RT}: Reaction total volume, 0.3 mL;

V_{S}: Sample volume, 0.05 mL;

V_{ST}: Extract solution volume, 1 mL;

Cpr: Sample protein concentration, mg/mL;

W: Sample weight, g;

500: The amount of bacteria or cells, 5 million;

T: Reaction time, 10 minutes.

Note:

Different sample of PPO has different optimum reaction temperature, adjust temperature at 25-37℃.

Recent Product Citations:

[1] Zheng XR, Zhang MJ, Qiao YH, Li R, Alkan N, Chen JY, Chen FM. Cyclocarya paliurus Reprograms the Flavonoid Biosynthesis Pathway Against Colletotrichum fructicola. Front Plant Sci. 2022 Jun 30; 13:933484. doi: 10.3389/fpls.2022.933484. PMID: 35845688; PMCID: PMC9280340.

[2] Finzi AC, Abramoff RZ, Spiller KS, Brzostek ER, Darby BA, Kramer MA, Phillips RP. Rhizosphere processes are quantitatively important components of terrestrial carbon and nutrient cycles. Glob Chang Biol. 2015 May;21(5):2082-94. doi: 10.1111/gcb.12816. Epub 2015 Jan 28. PMID: 25421798.

[3] Xu W, Yang Q, Yang F, Xie X, Goodwin PH, Deng X, Tian B, Yang L. Evaluation and Genome Analysis of Bacillus subtilis YB-04 as a Potential Biocontrol Agent Against Fusarium Wilt and Growth Promotion Agent of Cucumber. Front Microbiol. 2022 Jun 9; 13:885430. doi: 10.3389/fmicb.2022.885430. PMID: 35756052; PMCID: PMC9218633.

[4] Shang XC, Cai X, Zhou Y, Han X, Zhang CS, Ilyas N, Li Y, Zheng Y. Pseudomonas Inoculation Stimulates Endophytic Azospira Population and Induces Systemic Resistance to Bacterial Wilt. Front Plant Sci. 2021 Sep 22; 12:738611. doi: 10.3389/fpls.2021.738611. PMID: 36406638; PMCID: PMC9673043.

For research use only. Do not use for clinical, diagnostic, food, cosmetic testing and other purposes.

[5] Dong Q, Liu Q, Goodwin PH, Deng X, Xu W, Xia M, Zhang J, Sun R, Wu C, Wang Q, Wu K, Yang L. Isolation and Genome-Based Characterization of Biocontrol Potential of Bacillus siamensis YB-1631 against Wheat Crown Rot Caused by Fusarium pseudograminearum. J Fungi (Basel). 2023 May 9;9(5):547. doi: 10.3390/jof9050547. PMID: 37233258; PMCID: PMC10219336.

References:

[1] González, Eva M, De Ancos B, Cano M P. Partial Characterization of Polyphenol Oxidase Activity in Raspberry Fruits[J]. Journal of Agricultural and Food Chemistry, 1999, 47(10):4068-4072.

[2] Hong - Wei Zhou, Feng X . Polyphenol oxidase from yali pear (Pyrus bretschneideri) [J]. Journal of the Science of Food & Agriculture, 1991, 57(3):307-313.

[3] Tang W, Newton R J. Increase of polyphenol oxidase and decrease of polyamines correlate with tissue browning in Virginia pine (Pinus virginiana Mill.) [J]. plant science, 2004, 167(3):621-628.

Related Products:

BC0210/BC0215	Phenylalnine Ammonialyase (PAL) Activity Assay Kit
BC0170/BC0175	Superoxide Dismutase(SOD) Activity Assay Kit
BC0200/BC0205	Catalase(CAT) Activity Assay Kit
BC0090/BC0095	Peroxidase(POD) Activity Assay Kit