Polyphenol Oxidase (PPO) Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Catalog Number: BC0190

Size:50T/24S

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 30 mL×1	2-8℃
Powder I	Powder×1	2-8℃
Reagent I	Liquid 40 mL×1	2-8℃
Reagent II	Liquid 10 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.

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, refrigerated centrifuge, water bath, transferpettor, mortar/homogenizer/cell ultrasonic crusher, 1 mL glass cuvette, 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. Centrifuge before detect if there are precipitation.

II. Determination procedure.

1. Preheat spectrophotometer for 30 minutes, adjust wavelength to 410 nm and set counter to zero with

distilled water.

2. Add reagents with the following list:

Reagent (μL)	Test tube (T)	Contrast tube (C)
Reagent I	600	600
Reagent II	150	150
Sample	150	-
Boiled sample	-	150
Incubate at 37℃ (mammals) or 25℃ (other species) water bath for 10 minutes. Heat in boiled water for 10 minutes (Wrap the sealing film to prevent bursting). After cooling, centrifuge at 5000 ×g for 10 minutes at room temperature, take the supernatant. Then 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.

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 number:

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)= $\Delta A \div 0.01 \times V_{RT} \div (500 \div V_{ST} \times V_S) \div T = 0.12 \times \Delta A$

V_{RT}: Reaction total volume, 0.9 mL;

V_S: Sample volume, 0.15 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] Wang Y, Yang L, Zhou X, Wang Y, Liang Y, Luo B, Dai Y, Wei Z, Li S, He R, Ding W. Molecular mechanism of plant elicitor daphnetin-carboxymethyl chitosan nanoparticles against Ralstonia solanacearum by activating plant system resistance. Int J Biol Macromol. 2023 Jun 30; 241:124580. doi: 10.1016/j.ijbiomac.2023.124580. PMID: 37100321.

[2] Wang X, Zhang X, Jia P, Luan H, Qi G, Li H, Guo S. Transcriptomics and metabolomics provide insight into the anti-browning mechanism of selenium in freshly cut apples. Front Plant Sci. 2023 May 8;14:1176936. doi: 10.3389/fpls.2023.1176936. PMID: 37223812; PMCID: PMC10200898.

[3] Dai Y, Xie H, Zhao X, Zheng Y. The Effect of Sodium Nitroprusside Treatment on Storage Ability of Fresh-Cut Potato. Foods. 2023 Jan 3;12(1):221. doi: 10.3390/foods12010221. PMID: 36613434; PMCID: PMC9818613.

[4] Luo SZ, Sun Y, Yuan X, Pan LH, Zheng Z, Zhao YY, Zhong XY. Infrared radiation blanching-inhibited browning and extended shelf life of pecan kernels. J Food Sci. 2023 Apr;88(4):1566-1579. doi: 10.1111/1750-3841.16505. Epub 2023 Feb 16. PMID: 36798018.

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