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Polyphenol Oxidase (PPO) Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Cat No: AK0293

Size:50T/24S

Components:

Extract solution: Liquid 60 mL×1, store at 4℃ . Mix thoroughly with Powder I before use.

Powder I: Powder×1, store at 4℃ .

Reagent I: Liquid 40 mL×1, store at 4℃ .

Reagent II: Liquid 10 mL×1, store at 4℃ .

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, 1 mL glass cuvette, mortar/ homogenizer, 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.

II. Determination procedure.

1. Preheat spectrophotometer for 30 minutes, adjust wavelength to 410 nm, set 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. 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 (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 (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 (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] Li B, Ding Y, Tang X, et al. Effect of L-Arginine on Maintaining Storage Quality of the White Button Mushroom (Agaricus bisporus) [J]. Food and Bioprocess Technology, 2019, 12(4): 563-574.

[2] B Li, Y Ding, X Tang, et al. MTA1 promotes the invasion and migration of pancreatic cancer cells potentially through the HIF-α/VEGF pathway. Journal of Receptor and Signal Transduction Research. August 2018;(IF2.998)

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:

AK0578/AK0577 Phenylalnine Ammonialyase (PAL) Activity Assay Kit
AK0584/AK0583 Superoxide Dismutase(SOD) Activity Assay Kit
AK0580/AK0579 Catalase(CAT) Activity Assay Kit
AK0598/AK0597 Peroxidase(POD) Activity Assay Kit