Tyrosinase Activity Assay Kit

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

Operation Equipment: spectrophotometer

Cat No: BC4050

Size:50T/48S

Components:

Reagent Name	Size	Storage
Extract solution	Liquid 110 mL×1	2-8℃
Reagent I	Powder×3	2-8℃
Reagent II	Liquid 9 mL×1	2-8℃
Reagent III	Powder×1	2-8℃

Reagent I: Dissolve thoroughly with 15 ml extract solution before use. Prepared when the solution will be used. Reagents should be used up as soon as possible within 24 hours.

Reagent III: Before use, add 3 mL of distilled water to fully dissolve for use, the reagent is a saturated solution, if precipitation can be placed in a 45 ℃ water bath heating to dissolve; inexhaustible reagent 2-8 ℃ storage for 4 weeks.

Product Description:

Tyrosinase(tyrosinase : EC1.14.18.1) is a monophenol monooxygenase, which is copper-containing glycoprotein with double functions and exists widely in plant, yeast and animal tissues. Tyrosinase is the key enzyme for synthesis of melanin, which is also the main factor for browning of fruits and vegetables, and have a key influence on immunity and growth of insects.

Tyrosinase catalyzes the formation of dopaquinone from L-dopa, which produces a purplish-red substance with MBTH, with a characteristic absorption peak at 505 nm, from which the tyrosinase activity can be calculated

Reagents and Equipment Required but Not Provided:

Spectrophotometer, cryogenic centrifuge, water bath/incubator, cell sonicator, adjustable pipette, 1mL glass cuvette, mortar/homogenizer/Cell Ultrasonic Crusher, ice, distilled water.

Sample preparation:

1. Tissue: The mass of tissue (g): the volume of Reagent I (mL) = 1:5~10 (it is recommended to weigh about 0.1 g of tissue, add 1 mL of Reagent I. Homogenate in ice bath. Centrifuge at 12000 ×g for 10 minutes at 4℃, take the supernatant and put it on ice for test.

2. Cells:

The number of cells (10^4): the volume of Reagent I (mL) is 500~1000:1 (it is recommended to add 1 mL of Reagent I to 5 million cells), the cells are broken by ice bath ultrasound (Power: 300 w,

ultrasound: 3 s, interval: 7 s, 30 times). Centrifuge at 12000 ×g for 10 minutes at 4℃, take the supernatant and put it on ice for test.

3. Liquid: Direct detection.

Procedure:

1. Preheat spectrophotometer for 30min, adjust the wavelength to 475 nm, set the counter to zero with distilled water.
2. Add the following reagents to 1ml glass cuvette:

Reagent Name（μL）	Test Tube (A_T)	Blank Tube (A_B)
Reagent I	750	750
Reagent II	150	150
Sample	100	-
Stilled Water	-	100
Mix well and react accurately for 20 minutes at 37℃		
Reagent III	50	50
Mix thoroughly, centrifuge at 12000 ×g for 3 minutes at 4. Take the supernatant. The absorbance at 505 nm is measured and recorded as A_T and A_B respectively. Calculate $\Delta A_T = A_T - A_B$. Blank tube should only be measured 1-2 times.		

Calculation:

1. Protein concentration:

Unit definition: One unit of enzyme activity is defined as 0.01 change in absorbance at 505 nm per mg of protein per minute in the reaction system.

Tyrosinase (U/mg prot)= $\Delta A_T \times V_{RV} \div (V_S \times Cpr) \div 0.01 \div T \times F = 52.5 \times \Delta A_T \div Cpr \times F$

2. Sample weight:

Unit definition: One unit of enzyme activity is defined as 0.01 change in absorbance at 505 nm per g of sample weight per minute in the reaction system.

Tyrosinase (U/g)= $\Delta A_T \times V_{RV} \div (W \div V_E \times V_S) \div 0.01 \div T \times F = 52.5 \times \Delta A_T \div W \times F$

3. Cells or bacteria:

Unit definition: One unit of enzyme activity is defined as 0.01 change in absorbance at 505 nm per 10^6 of cells per minute in the reaction system. .

Tyrosinase (U/10^4 cell)= $\Delta A_T \times V_{RV} \div (N \div V_E \times V_S) \div 0.01 \div T \times F = 52.5 \times \Delta A_T \div N \times F$

4. Liquid volume:

Unit definition: One unit of enzyme activity is defined as 0.01 change in absorbance at 505 nm per mL of Liquid per minute in the reaction system. .

Tyrosinase (U/ml)= $\Delta A_T \times V_{RV} \div V_S \div 0.01 \div T \times F = 52.5 \times \Delta A_T \times F$

V_{RV}: total reaction volume, 1.05mL;

Vs: Sample volume, 0.1 mL;

Cpr: sample protein concentration (mg/mL);

T: Reaction time (min), 3 min;

W: Sample weight(g);

V_{EV}: Extraction volume, 1 mL;

N: Total number of cells or bacteria, a million as a unit.

F: Dilution times.

Note:

1. If ΔA>1, it is suggested to dilute sample with distilled water and measure again.

Related Products:

BC1310/BC1315 Total Antioxidant Capacity(T-AOC) Assay Kit

BC1430/BC1435 Thiol Content Assay Kit (Non-Protein Sample)

BC1370/BC1375 Total Mercapto(-SH) Content Assay Kit

Tel: 86-010-50973105 https://www.solarbio.net E-mail: info@solarbio.com