Tyrosinase Activity Assay Kit

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

Operation Equipment: Microplate Reader/ spectrophotometer

Cat No: BC4055

Size:100T/96S

Components:

Extract solution: 125mL×1. Storage at 4℃.

Reagent I: Powder×3. Storage at 4℃. Working solution: dissolve each tube thoroughly with 7 ml of extract solution before use. Prepared when the solution will be used.

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 l-dopa to form dopachrome, the activity of tyrosinase can be detected by dopachrome that has characteristic absorption at 475nm.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/ microplate reader, micro glass cuvette/96 well flat-bottom plate, water bath, low temperature centrifuge, adjustable transferpettor, mortar, ice and distilled water.

Sample preparation:

1. Tissue: for 0.1g of tissue add 1 ml extract solution, fully grinding on ice. centrifuge at 12000g and 4℃ for 20min, place the supernatant on ice and test soon.
2. Cell or microbial sample: collect cell or microbial sample to centrifuge tube, remove supernatant, suggested 5 million with 1mL of extract solution, splitting bacteria and cell with ultrasonication (ice bath, power 20%, work time 3s, interval 10s, for 30 times), centrifuge at 12000g and 4℃ for 20min, place the supernatant on ice and test soon.
3. Serum: Detect directly.

Procedure:

1. Preheat spectrophotometer/ microplate reader for 30min, adjust the wavelength to 475 nm, and set to zero with distilled water.
2. Add the following reagents to ultra-micro glass cuvette/96 well plate:

Reagent	Test tube(T)
Reagent I (μL)	180
Sample(μL)	20

Mix thoroughly, detect absorbance of 475nm at 10s(A1), and then put the cuvette and react solution to 37℃ (mammal), 25℃ (others) water bath for 3min quickly. If the microplate reader has temperature control function, please just adjust to 37℃ or 25℃. Take out and dry it quickly, detect the OD at 475nm for 190s and denote as A2, ΔA=A2-A1.

Calculation:

I. Ultra-micro glass cuvette

1. Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the generation of 1nmol of dopachrome per min every mg of tissue protein.

Tyrosinase (U/mg prot)= $\Delta A \div (\varepsilon \times d) \times Vrv \times 10^9 \div (Vs \times Cpr) \div T = 90.09 \times \Delta A \div Cpr$

2. Sample weight:

Unit definition: One unit of enzyme activity is defined the amount of enzyme that catalyzes the generation of 1nmol of dopachrome per min every gram tissue weight.

Tyrosinase (U/g)= $\Delta A \div (\varepsilon \times d) \times Vrv \times 10^9 \div (W \div Vsv \times Vs) \div T = 90.09 \times \Delta A \div W$

3. Cells or bacteria:

Unit definition: One unit of enzyme activity is defined the amount of enzyme that catalyzes the generation of 1nmol of dopachrome per min every 10^4 cell.

Tyrosinase (U/10^4 cell)= $\Delta A \div (\varepsilon \times d) \times Vrv \times 10^9 \div (500 \div Vsv \times Vs) \div T = 0.18 \times \Delta A$

4. Liquid volume:

Unit definition: One unit of enzyme activity is defined the amount of enzyme that catalyzes the generation of 1nmol of dopachrome per min every mL of serum.

Tyrosinase (U/ml)= $\Delta A \div (\varepsilon \times d) \times Vrv \times 10^9 \div Vs \div T = 90.09 \times \Delta A$

II. Microplate Reader

Refer to micro glass cuvette formula, change D-1cm to D-0.6cm

ε: dopachrome molar extinction coefficient, 3.7×10^4L/mol/cm;

d: light path of cuvette, 1cm;

Vrv: total reaction volume,2×10^{-4}L;

Vs: supernate volume (mL), 0.02 mL;

Cpr: sample protein concentration (mg/mL);

T: Reaction time (min), 3 min;

W: Sample weight(g);

Vsv: Extraction volume, 1 mL;

500: 500 $\times 10^4$cells.

Note:

1. If △A>0.3(spectrophotometer) or △A>0.2(microplate reader), dilute sample with extract solution and measure again. If △A value is too small, it is suggested to increase the enzymatic reaction time (5min or 10min) or increase sample volume.

2. Reagent 1 is oxidized easily after dissolved. So please use up as soon as possible.

Experimental Examples:

1. Take 0.1g of Echinochloa crusgalli and add 1mL extract to homogenize and grind, take the supernatant and operate according to the determination procedure, and calculate ΔA=A2-A1=0.105-0.054=0.051, use micro glass cuvette and calculate the enzyme activity according to the sample weight:
   Tyrosinase Activity（U/g weight）=90.09×ΔA÷W=90.09×0.051÷0.1=45.95U/g weight。
2. Take 0.1g of potatoes and add 1mL extract to homogenize and grind. Dilute the supernatant 4 times and follow the determination procedure. The measured calculation is ΔA=A2-A1=0.200-0.047=0.153, use micro glass cuvette and calculate the enzyme activity according to the sample weight:
   Tyrosinase Activity（U/g weight）=90.09×ΔA÷W×F（Dilute times）=90.09×0.153÷0.1×4=551.35U/g weight。
3. Take the rabbit serum, operate according to the determination procedure, calculate as ΔA=A2-A1=0.388-0.328=0.06, use micro glass cuvette and calculate the enzyme activity according to the sample volume:
   Tyrosinase Activity（U/mL）=90.09×ΔA=90.09×0.06=5.4 U/mL。

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