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℃. Dissolve thoroughly with 7 ml extract solution before use. Prepared when the solution will be used. Reagents should be used up as soon as possible within 24 hours.

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/plate reader, cryogenic centrifuge, water bath/incubator, cell sonicator, adjustable pipette, micro glass cuvettes/96-well plate, mortar/homogenizer, ice, 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 200w, 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. (If the solution is turbid, take the supernatant after centrifugation for measurement)

Procedure:

1. Preheat spectrophotometer/ microplate reader for 30min, adjust the wavelength to 475 nm, and spectrophotometer 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

Add the above reagents to the cuvette/96-well plate respectively, then pipette and mix quickly, record the absorbance value A1 at the 10s(A1), and quickly place it in a water bath or incubator at 37°C (mammals) or 25°C (other species) for 3 minutes. Take out and wipe it dry quickly to measure the absorbance value A2 at 3min10s, and calculate 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)= ΔA÷(ε×d)×Vrv×10^9÷(Vs×Cpr)÷T =90.09×ΔA÷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)= ΔA÷(ε×d)×Vrv×10^9÷(W÷Vsv×Vs)÷T=90.09×ΔA÷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)=ΔA÷(ε×d)×Vrv×10^9÷(500÷Vsv×Vs)÷T=0.18×ΔA

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)= ΔA÷(ε×d)×Vrv×10^9÷Vs÷T=90.09×ΔA

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 ×10^4cells.

Note:

1. If ΔA>0.3(spectrophotometer) or ΔA>0.2(microplate reader), dilute sample with extract solution and measure again. If ΔA value is too small, it is suggested to increase the enzymatic reaction time (5min or 10min) or increase sample volume. (At the time of calculation, pay attention to modify the calculation formula synchronously)

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=0.0821-0.0368=0.0453, use micro glass cuvette and calculate the enzyme activity according to the sample weight:
   Tyrosinase Activity（U/g weight）=90.09×ΔA÷W=90.09×0.0453÷0.1=40.81U/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=0.2021-0.0239=0.1782, 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）=90.09×0.1782÷0.1×4=642.16U/g weight。
3. Take the rabbit serum, operate according to the determination procedure, calculate as ΔA=A2-A1=0.2981-0.2556=0.0425, use micro glass cuvette and calculate the enzyme activity according to the sample volume:
   Tyrosinase Activity（U/mL）=90.09×ΔA=90.09×0.0425=3.83 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

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