Tyrosine Ammonia-Lyase (TAL) Activity Assay Kit

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

Operation Equipment: Ultraviolet spectrophotometer

Cat No: BC4060

Size:50T/48S

Components:

Extract solution: 60mL×1. Storage at 4℃.

Reagent I: 40mL×1. Storage at 4℃.

Reagent II: Powder×2. Storage at 4℃, take 1 bottle and add 5 mL of distilled water and 20 μL of concentrated HCl (37%) to fully dissolve it for use. Reagents need to be prepared and used immediately. Reagents stored at 4°C for 4 weeks

Product Description:

Tyrosine ammonia-lyase (TAL) existed widely in plants and microorganisms, is one of the key enzymes in the secondary metabolic pathway of phenylalanine. TAL can transform tyrosine into coumaric acid directly without cinnamic acid-4-hydroxylase (C4H). Coumaric acid can form phenylpropanoids natural products like resveratrol and naringin, which have an effect of antioxidant and anti-aging.

Tyrosine ammonia-lyase (TAL) decomposes tyrosine to from coumaric acid, which has absorbance at 310 nm. So the activity of TAL can be detected by the changing rate of absorbance.

Reagents and Equipment Required but Not Provided:

Ultraviolet spectrophotometer, cryogenic centrifuge, water bath/incubator, cell sonicator, adjustable pipette, 1mL quartz cuvette, mortar/homogenizer, ice, concentrated hydrochloric acid and distilled water.

Sample preparation:

1. Tissue: Add 1 ml of extract solution into 0.1g of tissue and fully grind on ice. centrifuge at 12000g and 4℃ for 10min, supernatant on ice is used for test.
2. Cells or bacterium: collect cells or bacterium to centrifuge and remove the supernatant. Suggested 5 million with 1mL of extract solution, split bacteria and cell with ultrasonication (power 200w, work time 3s, interval 10s, for 30 times). centrifuge at 12000g and 4℃ for 10min, supernatant on ice is used for test.

Procedure:

1. Preheat ultraviolet spectrophotometer for 30min, adjust the wavelength to 310 nm, set the counter to zero with ddH_2O.
2. Add the following reagents to 1ml quartz cuvette:

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Reagent name	Test tube (T)
Reagent I(μL)	700
Reagent II(μL)	200
Sample(μL)	100

Add the above reagents to the cuvettes respectively, then pipette and mix quickly, record the absorbance value A1 at 10s, quickly place them in a water bath or incubator at 37°C (mammals) or 25°C (other species) for 3 minutes, take them out and wipe them dry. Measure the absorbance value A2 at 3min10s, and calculate ΔA=A2-A1.

Calculation:

1. **Protein concentration:**

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that changes the absorbance of 0.01 at 310nm per min every milligram tissue protein.

TAL (U/mg prot) = ΔA÷0.01×Vrv÷(Vs×Cpr) ÷T =333×ΔA÷Cpr

2. **Sample weight:**

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that changes the absorbance of 0.01 at 310nm per min every gram tissue protein.

TAL (U/g) = ΔA÷0.01×Vrv÷(W÷Vsv×Vs) ÷T=333×ΔA÷W

3. **Cells or bacteria:**

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that changes the absorbance of 0.01 at 310nm per min per 10^4 cell or bacteria.

TAL (U/10^4 cell) =ΔA÷0.01×Vrv÷(500÷Vsv×Vs) ÷T=0.667×ΔA

Vrv: total reaction volume,1 mL;
Vs: supernatant volume (mL), 0.1 mL;
Cpr: sample protein concentration (mg/mL);
T: Reaction time (min), 3 min;
W: Sample weight(g);
Vsv: Extraction volume, 1 mL;
500: 5 million cells.

Note:

1. When ΔA is greater than 0.2 or A1 is greater than 1.5, it is recommended to dilute the sample with distilled water for measurement; if ΔA is too small, it is recommended to increase the enzymatic reaction time (5min or 10min) or increase the volume of the sample added for measurement. When calculating, pay attention to modify the calculation formula synchronously. (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 dilute 3 times and follow the determination procedure, the measured calculation is ΔA=A2-A1=0.253-0.243=0.01, calculated according to the sample weight:
   TAL（U/g weight）=333×ΔA÷W×F（dilute times）=333×0.01÷0.1×3=99.9 U/g weight。

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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