Tyrosine Ammonia-Lyase (TAL) Activity Assay Kit

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

Operation Equipment: Spectrophotometer /Microplate Reader

Cat No: BC4065

Size:100T/96S

Components:

Extract solution: 100mL×1. Storage at 4℃.

Reagent I: 20mL×1. Storage at 4℃.

Reagent II: Powder×2. Storage at 4℃, dissolve thoroughly with 2.8 mL of distilled water and 11 μL of concentrated hydrochloric acid before use.

Product Description:

Tyrosine ammonia-lyase (TAL) existed widely in plants and microorganisms, is one of the key of 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:

Spectrophotometer/ microplate reader, micro quartz cuvette/96 well flat-bottom UV plate, water bath, low temperature centrifuge, adjustable transferpettor, mortar, 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. centrifuged at 12000rpm and 4℃ for 10min, supernatant on ice is used for test.
2. Cells or microbial sample: collect cells or microbial sample to centrifuge and remove the supernatant. Suggested 5 million with 1mL of extract solution, split bacteria/cell with ultrasonication (power 20%, work time 3s, interval 10s, for 30 times). centrifuge at 12000rpm and 4℃ for 10min, supernatant on ice is used for test.

Procedure:

1. Preheat spectrophotometer or microplate Reader for 30min, adjust the wavelength to 310 nm, set the counter to zero with distilled water.
2. Add the following reagents to micro quartz cuvette/96 well UV plate:

Reagent name	Test tube (T)
Reagent I(μL)	140

Reagent II(μL)	40
Sample(μL)	20

Mix thoroughly, detect absorbance at 310nm for 10s, noted A1. Put the cuvette and react solution to 37℃ water bath for 3min (if the microplate reader has temperature control function, adjust the temperature to 37℃)., take out and dry it quickly, detect absorbance at 310nm for 190s, noted A2, calculate △A=A2-A1.

Calculation:

ultra-micro quartz cuvette:

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/104 cell)=ΔA÷0.01×Vrv÷(500÷Vsv×Vs)÷T=0.667×ΔA

Vrv: total reaction volume,0.2 mL;

Vs: supernatant 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: 5 million cells.

96 well plate:

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÷0.005×Vrv÷(Vs×Cpr) ÷T =667×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÷0.005×Vrv÷(W÷Vsv×Vs) ÷T=667×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 104 cell or bacteria.
TAL (U/104 cell) =ΔA÷0.005×Vrv÷(500÷Vsv×Vs) ÷T=1.334×ΔA

Vrv: total reaction volume,0.2 mL;
Vs: supernata
nt 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: 5 million cells.

Note:

1. Dilute sample with distilled water if △A>0.2 or A1>1.5. Increase the reacting time (5min or10min) and sample volume if △A is too low.

Experimental Examples:

1. Take 0.1g of Hibiscus Leaf and add 1mL extract to homogenize and grind, take the supernatant and dilute 3 times and follow the determination procedure, the measured calculation by ultra-micro quartz cuvette is ΔA=A2-A1=0.4813-0.4689=0.0124, calculated according to the sample weight:
   TAL（U/g weight）=333×ΔA÷W×F（dilute times）=333×0.0124÷0.1×3=123.876 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