

## 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°C.

**Reagent I:** 20mL×1. Storage at 4°C.

**Reagent II:** Powder×2. Storage at 4°C, take 1 bottle and add 2.8 mL of distilled water and 11 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 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:

Ultraviolet spectrophotometer / microplate reader, micro quartz cuvette/96 well flat-bottom UV plate, cryogenic centrifuge, water bath/incubator, cell sonicator, adjustable pipette, 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. centrifuged at 12000g and 4°C 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 200w, work time 3s, interval 10s, for 30 times). centrifuge at 12000g and 4°C for 10min, supernatant on ice is used for test.

### Procedure:

1. Preheat spectrophotometer or microplate Reader for 30min, adjust the wavelength to 310 nm, spectrophotometer 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

Add the above reagents to a micro-quartz cuvette/96-well UV plate respectively, then pipette and mix quickly, record the absorbance value A1 at 10s, and quickly place it in a water bath or incubator at 37°C (mammals) or 25°C (other species). 3min, take out and dry it quickly to measure the absorbance value A2 at 3min10s, and calculate  $\Delta A = A2 - A1$ .

### 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.

$$\text{TAL (U/mg prot)} = \Delta A \div 0.01 \times V_{rv} \div (V_s \times C_{pr}) \div T = 333 \times \Delta A \div C_{pr}$$

##### 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.

$$\text{TAL (U/g)} = \Delta A \div 0.01 \times V_{rv} \div (W \div V_{sv} \times V_s) \div T = 333 \times \Delta A \div W$$

##### 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<sup>4</sup> cell or bacteria.

$$\text{TAL (U/10}^4 \text{ cell)} = \Delta A \div 0.01 \times V_{rv} \div (500 \div V_{sv} \times V_s) \div T = 0.667 \times \Delta A$$

V<sub>rv</sub>: total reaction volume, 0.2 mL;

V<sub>s</sub>: supernatant volume (mL), 0.02 mL;

C<sub>pr</sub>: sample protein concentration (mg/mL);

T: Reaction time (min), 3 min;

W: Sample weight(g);

V<sub>sv</sub>: 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 0.005 at 310nm per min every milligram tissue protein.

$$\text{TAL (U/mg prot)} = \Delta A \div 0.005 \times V_{rv} \div (V_s \times C_{pr}) \div T = 667 \times \Delta A \div C_{pr}$$

##### 2. Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that changes the

absorbance of 0.005 at 310nm per min every gram tissue protein.

$$\text{TAL (U/g)} = \Delta A \div 0.005 \times V_{rv} \div (W \div V_{sv} \times V_s) \div T = 667 \times \Delta A \div W$$

### 3. Cells or bacteria:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that changes the absorbance of 0.005 at 310nm per min per 10<sup>4</sup> cell or bacteria.

$$\text{TAL (U/10}^4 \text{ cell)} = \Delta A \div 0.005 \times V_{rv} \div (500 \div V_{sv} \times V_s) \div T = 1.334 \times \Delta A$$

V<sub>rv</sub>: total reaction volume, 0.2 mL;

V<sub>s</sub>: supernatant volume (mL), 0.02 mL;

C<sub>pr</sub>: sample protein concentration (mg/mL);

T: Reaction time (min), 3 min;

W: Sample weight(g);

V<sub>sv</sub>: Extraction volume, 1 mL;

500: 5 million cells.

#### Note:

- When  $\Delta A$  is greater than 0.2 or A<sub>1</sub> is greater than 1.5, it is recommended to dilute the sample with distilled water for measurement; if  $\Delta A$  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. (At the time of calculation, pay attention to modify the calculation formula synchronously).

#### Experimental Examples:

- 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  $\Delta A = A_2 - A_1 = 0.4813 - 0.4689 = 0.0124$ , calculated according to the sample weight:

$$\text{TAL (U/g weight)} = 333 \times \Delta A \div W \times F \text{ (dilute times)} = 333 \times 0.0124 \div 0.1 \times 3 = 123.876 \text{ U/g weight.}$$

#### 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

