Technical Manual

Tyrosine Ammonia-lyase (TAL) Activity Assay Kit

- Catalogue Code: MAES0201
- Size: 96T
- Research Use Only

1. Key Features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Sensitivity:

0.12 U/mL

Storage:

2-8°C for 3 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 3 months.

Do not use components from different batches of kit.

2. Background

Tyrosine ammonia-lyase (TAL), an aromatic amino acid lyase, is one of the key enzymes in the phenylalanine metabolic pathway and widely exists in plants and microorganisms. TAL can directly convert L-tyrosine to 4-coumaric acid by non-oxidative deamination without cinnamate 4-hydroxylase (C4H). 4-Coumaric acid can further generate natural phenylpropanoids with antioxidant and anti-aging effects such as resveratrol, naringin, etc.

3. Intended Use

This kit can be used to measure tyrosine ammonia-lyase (TAL) activity in fruit juices, plant and animal tissue samples.

4. Detection Principle

TAL can decompose tyrosine to produce 4-coumaric acid, which has a strong absorption peak at 333 nm. Therefore, the activity of TAL can be calculated by measuring the OD value at 333 nm.

5. Kit Components & Storage

Item	Specification	Storage
Extracting Solution	50 mL × 1 vial	2-8°C, 3 months
Buffer Solution	35 mL × 2 vials	2-8°C, 3 months
Substrate	Lyophilized × 2 vials	2-8°C, 3 months
Stop Solution	1.5 mL × 2 vials	2-8°C, 3 months
UV Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Water bath
- centrifuge
- Microplate reader (323-343 nm)
- Tips (10 µL, 200 µL, 1000 µL)
- EP tubes (1.5 mL, 2 mL)

6. Assay Notes:

1. Homogenate and centrifuge at 2-8°C during the preparation of sample supernatant, preserve the supernatant on ice and detect within half a day.

2. Centrifuge sample supernatant for several times if it is turbidity.

7. Reagent Preparation:

1. Precool extracting solution at 2-8°C before use, and bring the other reagents to room temperature.

2. Preparation of **Substrate working solution:** Dissolve the substrate with 30 mL of buffer solution in 45°C water bath for more than 20 min and mix fully before use. If there is solid precipitation, can be dissolved in 45°C water bath before use. It can be stored 2-8°C for 1 week.

8. Sample Preparation

A crude enzyme solution (for sample tubes)

1. Liquid sample: Detect the sample directly.

2. Tissue sample:
Take 0.05-0.1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. Use filter paper to absorb excess water and weigh. Homogenize at the ratio of the volume of extracting solution (2-8°C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4°C. Take the supernatant and preserve on ice before detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

B crude enzyme solution (for control tubes)

Take liquid samples or 50% tissue supernatant to 1.5 mL EP tube, bathed at 100°C for 5 min, cooled with ice water, centrifuge at 10000 g for 10 min and used as supernatant for control tubes.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range.

The recommended dilution factor for different samples is as follows (for reference only).

Sample Type:	Dilution Factor:
20% Epipremnum aureum tissue homogenate	1
20% Celery tissue homogenate	1
20% Cilantro tissue homogenate	1
20% Rat liver tissue homogenate	1
20% Rat kidney tissue homogenate	1
20% Corn tissue homogenate	1

Note: The diluent is extracting solution.

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 333 nm

Plate Set Up:

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1′	S9	S9′	S17	S17′	S25	S25′	S33	S33′	S41	S41′
B	S2	S2′	S10	S10′	S18	S18′	S26	S26′	S34	S34′	S42	S42′
C	S3	S3′	S11	S11′	S19	S19′	S27	S27′	S35	S35′	S43	S43′
D	S4	S4′	S12	S12′	S20	S20′	S28	S28′	S36	S36′	S44	S44′
E	S5	S5′	S13	S13′	S21	S21′	S29	S29′	S37	S37′	S45	S45′
F	S6	S6′	S14	S14′	S22	S22′	S30	S30′	S38	S38′	S46	S46′
G	S7	S7′	S15	S15′	S23	S23′	S31	S31′	S39	S39′	S47	S47′
H	S8	S8′	S16	S16′	S24	S24′	S32	S32′	S40	S40′	S48	S48′

Note: S1-S48: Sample wells; S1 '-S48': control wells.

10. Operation Steps

1. **Sample tube:** Take 40 μL of A crude enzyme solution into1.5 mL EP tubes.
   Control tube: Take 40 μL of B crude enzyme solution into1.5 mL EP tubes.

2. Add 360 μL of substrate working solution into each tube.

3. Mix fully and incubate at 37°C for 45 min.

4. Add 20 μL of stop solution into each tube.

5. Mix fully, centrifuge at 10000 g at 4°C for 5 min and take 200 μL of supernatant to the corresponding wells. Measure the OD values of each well at 333 nm with UV microplate reader.

	Control tube	Sample tube
A crude enzyme solution (μL)		40
B crude enzyme solution (μL)	40	
Substrate working solution (μL)	360	360
Mix fully and incubate at 37°C for 45 min.		
Stop solution (μL)	20	20
Mix fully, centrifuge at 10000 g at 4°C for 5 min and take 200 μL of supernatant to the corresponding wells. Measure the OD values of each well at 333 nm with UV microplate reader.		

11. Calculations

1. Fruit juices sample:

Definition: The change about 0.005 of absorbance at 333 nm by 1 mL of juice per minute in the reaction system at 37°C is defined as 1 activity unit.

$$\text{TAL activity (U/mL)} = \Delta A \times V_1 \div V_2 \div 0.005 \div t$$

2. Tissue sample:

Definition: The change about 0.005 of absorbance at 333 nm by 1 g of wet weight per minute in the reaction system at 37°C is defined as 1 activity unit.

$$\text{TAL activity (U/g wet weight)} = \Delta A \times V_1 \div (m \times V_2 \div V_3) \div 0.005 \div t$$

ΔA: $(OD_{Sample} - OD_{Control})$;
V_1: The volume of the reaction, 0.42 mL;
V_2: The volume of the sample supernatant, 0.04 mL;
V_3: The volume of the homogenate, mL;
m: Weight of tissue, g;
T: The time of incubation, 45 min;

12. Performance Characteristics

Analysis

For epipremnum aureum tissue, take 0.1 g 20% of prepared epipremnum aureum supernatant and operate according to the operation table.

The results are as follows:

The average OD value of the control well is 0.278, and the average OD value of the sample well is 0.290. The calculated results are:

$$\text{TAL activity (U/g wet weight)} = (0.290 - 0.278) \times 0.42 \div (0.1 \times 0.04 \div 0.4) \div 0.005 \div 45$$

$$= 2.24 \text{ U/g wet weight}$$

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

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