



TECHNICAL MANUAL

Tyrosine Ammonia-lyase (TAL) Activity Assay Kit

- **SKU CODE:** MAES0201
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

1. Assay summary

- Reagent preparation
- Sample preparation
- Add sample and substrate working solution
- Incubate at 37°C for 45 min
- Add stop solution
- Measure OD at 333 nm

2. Intended use

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

3. 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.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extracting Solution	25 mL × 1 vial	50 mL × 1 vial	2-8°C, 12 months
Reagent 2	Buffer Solution	35 mL × 1 vial	35 mL × 2 vials	2-8°C, 12 months
Reagent 3	Substrate	Powder × 1 vial	Powder × 2 vials	2-8°C, 12 months
Reagent 4	Stop Solution	1.5 mL × 1 vial	1.5 mL × 2 vials	2-8°C, 12 months
	UV Microplate	96 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Micropipette, Incubator, Centrifuge, Microplate reader (323-343 nm, optimum wavelength: 333 nm), Water bath

6. Reagent preparation

1. Keep extracting solution at 2-8°C before use. Equilibrate other reagents to room temperature before use.
2. **Preparation of substrate working solution:** Dissolve one vial of substrate with 30 mL of buffer solution in 45°C water bath for more than 20 min and mix thoroughly before use. If there is solid precipitation, it can be dissolved in 45°C water bath before use. Store at 2-8°C for 1 week.

7. Sample preparation

Sample preparation

A. Crude enzyme solution (for sample tubes)

Serum and plasma: Detect directly. If not detected on the same day, the serum or plasma can be stored at -80°C for a month.

Tissue sample:

1. Harvest the amount of tissue needed for each assay (initial recommendation 50 mg).
2. Wash tissue in cold PBS (0.01 M, pH 7.4).
3. Homogenize 50 mg tissue in 200 μ L extracting solution with a dounce homogenizer at 4°C.
4. Centrifuge at 10,000 $\times$ g for 25 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection.

B. Crude enzyme solution (for control tubes)

Take liquid samples or 50% tissue supernatant to 1.5 mL EP tube, bathe at 100°C for 5 min, cool with ice water, centrifuge at 10,000 $\times$ g for 10 min and use the supernatant for control tubes.

Dilution of sample

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. For the dilution of other sample types, please perform a pre-test to confirm the dilution factor.

8. The key points of the assay

1. Homogenize 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 several times if it is turbid.

9. Operating steps

1. Sample tube: Take 40 µL of A crude enzyme solution into 1.5 mL EP tubes. Control tube: Take 40 µL of B crude enzyme solution into 1.5 mL EP tubes.
2. Add 360 µL of substrate working solution into each tube.
3. Mix thoroughly and incubate at 37°C for 45 min.
4. Add 20 µL of stop solution into each tube.
5. Mix thoroughly, centrifuge at 10,000×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.

10. Calculation

The sample:

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.

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

[Note]

ΔA : $OD_{\text{Sample}} - OD_{\text{Control}}$.

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.

11. Appendix I Performance Characteristics

Sensitivity

The analytical sensitivity of the assay is 0.12 U/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

12. Appendix II Example Analysis

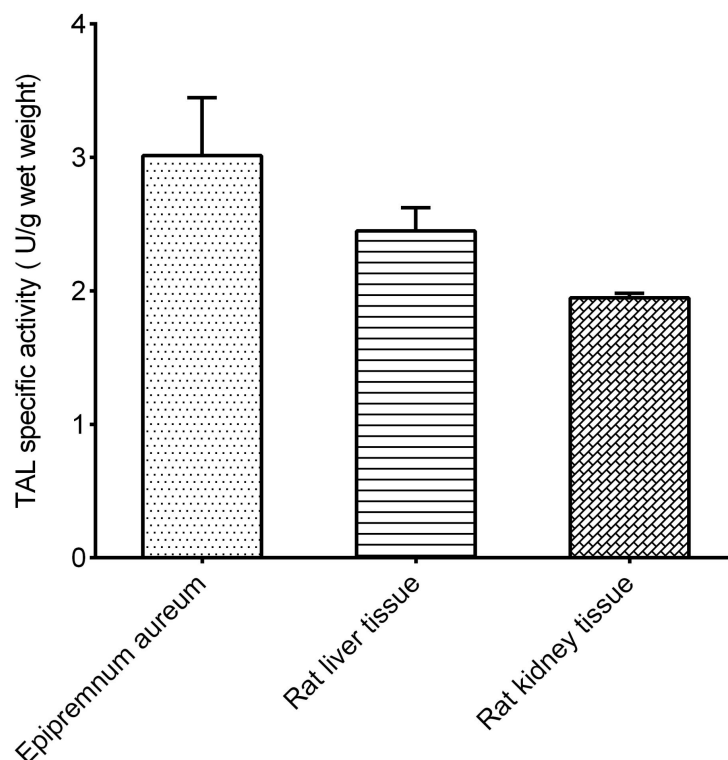
Example analysis:

For epipremnum aureum tissue, take 0.1 g 20% of prepared epipremnum aureum supernatant and operate according to the operation steps. 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, and the calculation result is:

$$\begin{aligned} \text{TAL activity (U/g tissue wet weight)} &= (0.290 - 0.278) \times 0.42 \div (0.1 \times 0.04 \div 0.4) \div 0.005 \div 45 \\ &= 2.24 \text{ U/g tissue wet weight} \end{aligned}$$

Detect 20% epipremnum aureum tissue homogenate, 20% rat liver tissue homogenate and 20% rat kidney tissue homogenate according to the protocol, the result is as follows:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.
6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

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Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.