（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K851-M

Specification：48T(24 samples)/96T(48 samples)

Measuring instrument：Microplate reader（323-343 nm）

Sensitivity: 0.12 U/mL

Elabscience®Tyrosine Ammonia-lyase (TAL) Activity Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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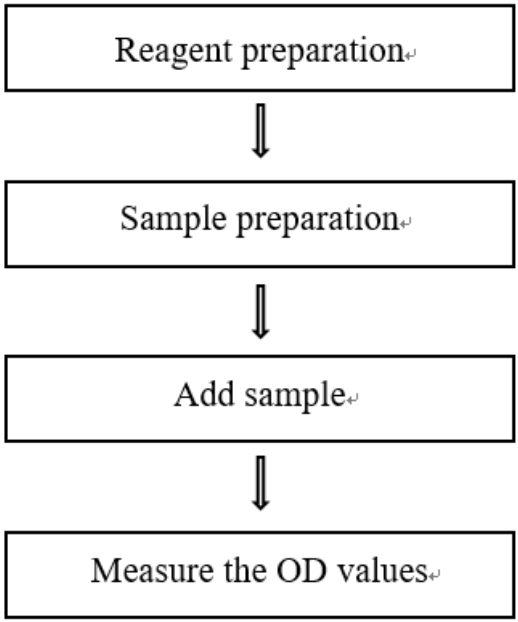
Assay summary



Intended use

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

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.

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℃, 12 months
Reagent 2	Buffer Solution	35 mL ×1 vial	35 mL ×2 vials	2-8℃, 12 months
Reagent 3	Substrate	Powder ×1 vial	Powder ×2 vials	2-8℃, 12 months
Reagent 4	Stop Solution	1.5 mL ×1 vial	1.5 mL ×2 vials	2-8℃, 12 months
	UV Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

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

Reagent preparation

① Keep extracting solution at 2-8 ℃ 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 ℃ water bath for more than 20 min and mix fully before use. If there is solid precipitation, can be dissolved in 45 ℃ water bath before use. Store at 2-8 ℃ for 1 week.

Sample preparation

① 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℃ 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 μL extracting solution with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 25 min at 4℃ 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, bathed at 100 ℃ for 5 min, cooled with ice water, centrifuge at 10000×g for 10 min and used as supernatant for control tubes.

② 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 do pretest to confirm the dilution factor.

The key points of the assay

① Homogenate and centrifuge at 2-8℃ 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.

Operating 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℃ for 45 min.

④ Add 20 μL of stop solution into each tube.

⑤ Mix fully, centrifuge at 10000×g at 4℃ 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.

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 oC 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 oC 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: $OD_{Sample} - OD_{Control}$.

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.

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.

Appendix II Example Analysis

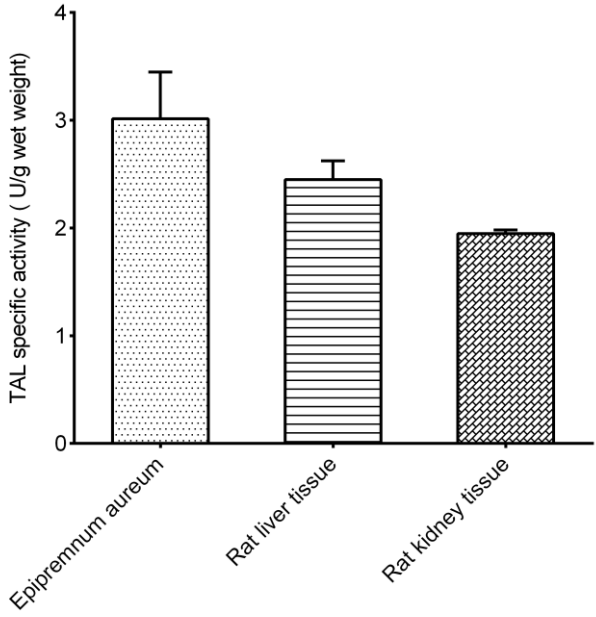
Example analysis:

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:

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

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

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:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.