PRODUCT INFORMATION & MANUAL

Tyrosine Ammonia Lyase/ TAL Activity Assay Kit (Colorimetric)

NBP3-25886

For research use only.
Not for diagnostic or therapeutic procedures.

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Tyrosine Ammonia Lyase/TAL

Activity Assay Kit (Colorimetric)

Catalog No: NBP3-25886

Method: Colorimetric method

Specification: 96T (Can detect 48 samples without duplication)

Instrument: Microplate reader

Sensitivity: 0.12 U/mL

▲ This kit is for research use only.

▲ Instructions should be followed strictly, changes of operation may result in unreliable results.

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

General information

▲Intended use

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

▲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.

▲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	Specification	Storage
Reagent 1	Extracting Solution	50 mL × 1 vial	2-8℃, 12 months
Reagent 2	Buffer Solution	35 mL × 2 vials	2-8℃, 12 months
Reagent 3	Substrate	Powder × 2 vials	2-8℃, 12 months
Reagent 4	Stop Solution	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.			

▲Materials prepared by users

Instruments

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

Reagents

Double distilled water

▲Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the 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.

▲Precautions

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

▲The key points of the assay

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.

Pre-assay preparation

▲Reagent preparation

1. Precool reagent 1 at 2-8°C before use, and bring the other reagents to room temperature.
2. Preparation of reagent 3 working solution:
   Dissolve the reagent 3 with 30 mL of reagent 2 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.

▲Sample preparation

1. A crude enzyme solution (for sample tubes)

Liquid sample: Detect the sample directly.

Tissue sample: Weigh the tissue 0.05-0.1 g, Add reagent 1 according to the ratio of weight (g): volume (mL)= 1:4, homogenate, centrifuge at 10000 g at 2-8°C for 25 min (if the sample supernatant is turbidity, centrifuge for several times), collect the supernatant and place it on ice for detection in half a day.

2. 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.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment.

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 reagent 1.

Assay protocol

▲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.

▲Detailed 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 reagent 3 working solution into each tube.

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

(4) Add 20 μL of reagent 4 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.

▲Summary operation table

	Control tube	Sample tube
A crude enzyme solution (μL)		40
B crude enzyme solution (μL))	40	
Reagent 3 working solution (μL)	360	360
Mix fully and incubate at 37°C for 45 min.		
Reagent 4 (μL)	20	20
Mix fully, centrifuge and take 200 μL of supernatant to the corresponding wells. Measure the OD values at 333 nm.		

▲Calculation

1) TAL activity in 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) AL activity in 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: $(OD_{Sample} - OD_{Control})$;

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;

▲Example 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:

TAL activity (U/g tissue wet weight)

= (0.290-0.278) × 0.42 ÷ (0.1 × 0.04 ÷ 0.4) ÷ 0.005 ÷ 45

= 2.24 U/g tissue wet weight

Appendix I References

1. Kazuko U, Hideaki Y, Tatsurokuro T, et al. Enzymatic Determination of L-Phenylalanine and L-Tyrosine [J]. Agricultural and Biological Chemistry, 1968(6):6.

2. Beaudoin-Eagan L D, Thorpe T A. Tyrosine and Phenylalanine Ammonia Lyase Activities during Shoot Initiation in Tobacco Callus Cultures 1 [J]. Plant Physiology, 1985(3):3.