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CheKine™ Micro Tyrosine Ammonia Lyase (TAL) Activity Assay Kit

Cat #: KTB1161 Size: 48 T/24 S 96 T/48 S

	Micro Tyrosine Ammonia Lyase (TAL) Activity Assay Kit		
REF	**Cat #:** KTB1161	LOT	**Lot #:** Refer to product label
	Applicable sample: Animal and Plant Tissues, Cells or Bacteria, Plasma, Serum or other Liquid samples		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Tyrosine ammonia lyase (TAL) is widely present in plants and microorganisms, and is one of the key enzymes in the phenylalanine metabolism pathway. It can directly convert tyrosine to coumaric acid by jumping over cinnamate-4-hydroxylase (C4H), which can further produce natural phenylalanine products such as resveratrol and naringenin, which have antioxidant and anti-aging effects. TAL decomposes tyrosine to produce coumaric acid, causing the absorbance of the reaction solution at 333 nm to increase with reaction time. The activity of TAL can be calculated based on the rate of change in absorbance.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	60 mL	120 mL	4℃
Reagent Ⅰ	30 mL	60 mL	4℃
Reagent Ⅱ	Powder×1 vial	Powder×2 vials	4℃, protected from light
Reagent Ⅲ	1.5 mL	3 mL	4℃

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 333 nm
- 96-well UV plate or microquartz cuvette, precision pipettes, disposable pipette tips
- Thermostatic water bath, ice maker, centrifuge, incubator
- Deionized water
- Mortar or homogenizer

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Note: Extraction Buffer is toxic and has a pungent odor, so it is recommended to experiment in a fume hood.

Reagent I: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent II: Prepared before use. Take a bottle of Reagent II and add 14 mL of Reagent I to it. Dissolve it thoroughly and soak it in water at 37℃ (for mammals) or 25℃ (for other species) for at least 10 min; Unused reagents can be stored in the dark at 4℃ for a week.

Reagent III: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month. When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

1. Tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Cells or Bacteria: Collect 5×10^6 cells or bacteria into the centrifuge tube, wash cells or bacteria with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cells or bacteria 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
3. Plasma, Serum or other Liquid samples: Test directly.

Note: It will be better to quantify the total protein with Protein Quantification Kit (Bradford Assay), Cat #: KTD3002, if it is calculated by protein concentration.

Assay Procedure

1. Preheat the microplate reader or ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 333 nm, ultraviolet spectrophotometer was returned to zero with deionized water.
2. Enzymatic reaction (The following operations are operated in the 1.5 mL EP tube):

Reagent	Test Tube (μL)	Control Tube (μL)
Sample supernatant	40	40
Reagent I	0	360
Reagent II	360	0
Mix thoroughly and incubate at 40℃ for 60 min		
Reagent III	20	20

Mix well, then 10,000 g, centrifuge at 4℃ for 5 min, take 200 μL of supernatant and transfer it to a microquartz cuvette or a 96 well UV plate, and measure the absorbance value at 333 nm. The absorbance of test tube, control tube were recorded as A_{Test}, $A_{Control}$. Calculate $\Delta A=A_{Test}-A_{Control}$.

Note: Each measuring tube needs to be equipped with a control tube. Before the experiment, it is suggested that 2-3 samples with large expected differences should be selected for pre-experiment. If ΔA is less than 0.4, the sample volume can be appropriately increased. If ΔA is greater than 2.0, the sample can be further diluted with Extraction Buffer before proceeding with the experiment, and the final dilution factor should be taken into account in the calculations.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Calculated by protein concentration

Active unit definition: The change in absorbance value at 333 nm by 0.005 per min per mg of tissue protein in the reaction system is defined as a unit of enzyme activity.

TAL (U/mg prot)=ΔA×V_{Total}÷(Cpr×V_{Sample})÷0.005÷T**=35×ΔA÷Cpr**

2. Calculated by sample fresh weight

Active unit definition: The change in absorbance value at 333 nm by 0.005 per min per g of tissue in the reaction system is defined as a unit of enzyme activity.

TAL (U/g fresh weight)=ΔA×V_{Total}÷(W×V_{Sample}÷$V_{Total\ Sample}$)÷0.005÷T**=35×ΔA÷W**

3. Calculated by number of cells or bacteria

Active unit definition: The change in absorbance value at 333 nm by 0.005 per min per 10^4 bacteria or cells in the reaction system is defined as a unit of enzyme activity.

TAL (U/g 10^4)=ΔA×V_{Total}÷(500×V_{Sample}÷$V_{Total\ Sample}$)÷0.005÷T**=0.07×ΔA**

4. Calculated by sample volume

Active unit definition: The change in absorbance value at 333 nm by 0.005 per min per mL serum (lasma) in the reaction system is defined as a unit of enzyme activity.

TAL (U/mL)=ΔA×V_{Total}÷V_{Sample}÷0.005÷T**=35×ΔA**

V_{Total}: total volume of enzymatic reaction, 0.42 mL; V_{Sample}: sample volume added, 0.04 mL; $V_{Total\ Sample}$: Extraction Buffer volume added, 1 mL; Cpr: sample protein concentration, mg/mL; T: reaction time, 60 min; W: sample weight, g; 2000: Total number of bacteria or cells, 5×10^5.

Typical Data

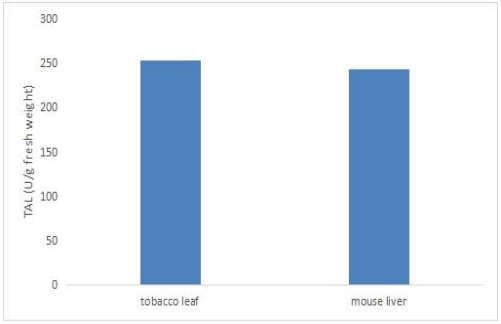

Figure 1. Determination TAL activity in tobacco leaf and mouse liver by this assay kit

Recommended Products

Catalog No.	Product Name
KTB1015	CheKine™ Micro α-Glucosidase Activity Assay Kit
KTB1121	CheKine™ Pyruvate Acid (PA) Colorimetric Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.