CheKine™ Micro Trehalase (THL) Activity Assay Kit

Cat #: KTB1336 Size: 48 T/96 T

	Micro Trehalase (THL) Activity Assay Kit		
REF	**Cat #:** KTB1336	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal and Plant Tissue, Cells or Fungus, Plasma, Serum or other Liquid samples		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Trehalase (THL) (EC3.2.1.28) is widely found in animals, plants, microorganisms and cultured cells. The main function of trehalase is that the organism breaks down trehalose to produce glucose, which is directly used for energy supply. CheKine™ Micro Trehalase (THL) Activity Assay Kit can be used to detect biological samples such as animal and plant tissue, cells or fungus, plasma, serum or other liquid samples. In the kit, THL catalyzes trehalose to produce glucose, glucose oxidase catalyzes glucose oxidation to gluconic acid, and hydrogen peroxide. Peroxidase catalyzes hydrogen peroxide to oxidize 4-amino-antipyrine conjugated phenol to produce colored compounds with characteristic absorption peaks at 505 nm, reflecting THL activity.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Reagent Ⅰ	1	1	4℃, protected from light
Reagent Ⅱ	7.5 mL	15 mL	4℃
Reagent Ⅲ	1	1	-20℃, protected from light
Reagent Ⅳ	5 mL	10 mL	4℃, protected from light
Standard	1	1	4℃, protected from light

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 505 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips, 1.5 mL EP tube
- Incubator, ice maker, freezing centrifuge
- Deionized water

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• Homogenizer or mortar (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.
Working Reagent Ⅰ: Prepared before use. Add 3 mL deionized water for 48 T and 6 mL deionized water for 96 T to fully dissolve. The prepared reagent can be stored at 4℃, protected from light for 2 week.
Reagent Ⅱ: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.
Working Reagent Ⅲ: Prepared before use. Add 5 mL Reagent Ⅱ for 48 T and 10 mL Reagent Ⅱ for 96 T to fully dissolve. The remaining reagent can also be stored at -20℃ and protected from light for 1 month after aliquoting to avoid repeated freezing and thawing.
Reagent Ⅳ: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃, protected from light.
Working Reagent: For each well, prepare 180 μL Working Reagent by mixing Reagent Ⅱ and Working Reagent Ⅲ in a 1: 1 volume. Working Reagent is freshly prepared.
Standard: Prepared before use; Add 1 mL deionized water to fully dissolve, that is 10 mg/mL glucose standard; Store at 4℃, protected from light for 2 weeks. Use the 10 mg/mL glucose standard solution and further dilute it to the standard as shown in the following table:

Num.	Standard Volume (μL)	Deionized Water (μL)	Concentration (mg/mL)
Std.1	100 μL of 10 mg/mL Standard	900	1
Std.2	160 μL of Std.1 (1 mg/mL)	40	0.8
Std.3	120 μL of Std.1 (1 mg/mL)	80	0.6
Std.4	80 μL of Std.1 (1 mg/mL)	120	0.4
Std.5	40 μL of Std.1 (1 mg/mL)	160	0.2
Blank	0	200	0

Notes: Always prepare fresh Standards per use; Diluted Std. solution is unstable and must be used within 4 h.

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 or mortar on ice. Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay.
2. Cells or Fungus: Collect 5×10^{6} cells or fungus into the centrifuge tube, wash cells or fungus with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cells or fungus 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.
3. Plasma, Serum or other Liquid samples: Take 0.1 mL liquid, add 1 mL Extraction Buffer and homogenize or mortar on ice. Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay.

Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 505 nm. Visible spectrophotometer was returned to zero with deionized water.

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2. Working Reagent place at 37°C (mammal) or 25°C (other species) incubation for 10 min.

3. Sample measurement. (The following operations are operated in the 1.5 mL EP tube)

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)	Control Well (μL)
Sample	0	0	30	30
Standard	0	30	0	0
Deionized Water	30	0	0	50
Working Reagent Ⅰ	50	50	50	0
After the accurate reaction in 45℃ water bath for 15 min, the reaction was terminated in 95℃ water bath for 5 min, and the supernatant can be obtained after 10,000 g centrifugation at 4℃ for 10 min. The following operations are operated in the 96-well plate or microglass cuvette:				
Supernatant	20	20	20	20
Working Reagent	180	180	180	180

4. Mix well, place at 37°C (mammal) or 25°C (other species) incubation for 15 min. Detect the absorbance at 505 nm. The Blank Well is recorded as A_{Blank}, the Standard Well is marked as $A_{Standard}$, the Test Well is marked as A_{Test}, the Control Well is marked as $A_{Control}$. Finally calculate $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: The Blank Well and the Standard Well only need to be done 1-2 times. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.05, increase the sample quantity appropriately. If ΔA_{Test} is greater than 1.0, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

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. Drawing of standard curve

With the concentration of the standard solution as the x-axis and the $\Delta A_{Standard}$ as the y-axis, draw the standard curve and obtain the standard equation. The determination of ΔA_{Test} is brought into the equation to get x (mg/mL).

2. Calculation of the THL activity

(1) Calculated by protein concentration

Active unit definition: 1 μg of glucose is produced per min in 1 mg tissue protein reaction system is defined as a unit of enzyme activity.

THL (U/mg prot)=$(V_{Reaction}\times x)\div(V_{Sample}\times Cpr)\div T\times 1{,}000$=**177.78x÷Cpr**

(2) Calculated by fresh weight of samples

Active unit definition: 1 μg of glucose is produced per min in 1 g tissue reaction system is defined as a unit of enzyme activity.

THL (U/g fresh weight)=$(V_{Reaction}\times x)\div(W\times V_{Sample}\div V_{Total\ sample})\div T\times 1{,}000$=**177.78x÷W**

(3) Calculated by fungus or cells

Active unit definition: 1 μg of glucose is produced per min in 10^4 fungus or cells reaction system is defined as a unit of enzyme activity.

THL (U/10^4)=$(V_{Reaction}\times x)\div(n\times V_{Sample}\div V_{Total\ sample})\div T\times 1{,}000$=**177.78x÷n**

(4) Calculated by volume of liquid samples

Active unit definition: 1 μg of glucose is produced per min in 1 mL Serum (Plasma) reaction system is defined as a unit of enzyme activity.

THL (U/mL)=$(V_{Reaction}\times x)\div(V_{Liquid}\times V_{Sample}\div V_{Total\ sample})\div T\times 1{,}000$=**1,777.78x**

$V_{Reaction}$: Added the sample volume, 80 μL=0.08 mL; V_{Sample}: Added the sample volume, 30 μL=0.03 mL; $V_{Total\ sample}$: Added the Extraction Buffer volume, 1 mL; V_{Liquid}: Added the plasma, serum or other liquid samples volume, 0.1 mL; T: Reaction time. 15

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min; Cpr: sample protein concentration, mg/mL; W: Sample weight, g; 1,000: 1 mg/mL=1,000 μg/mL; n: Number of fungus or cells, calculated in units of ten thousand.

Typical Data

The following data are for reference only. And the experimenters need to test the samples according to their own experiments.

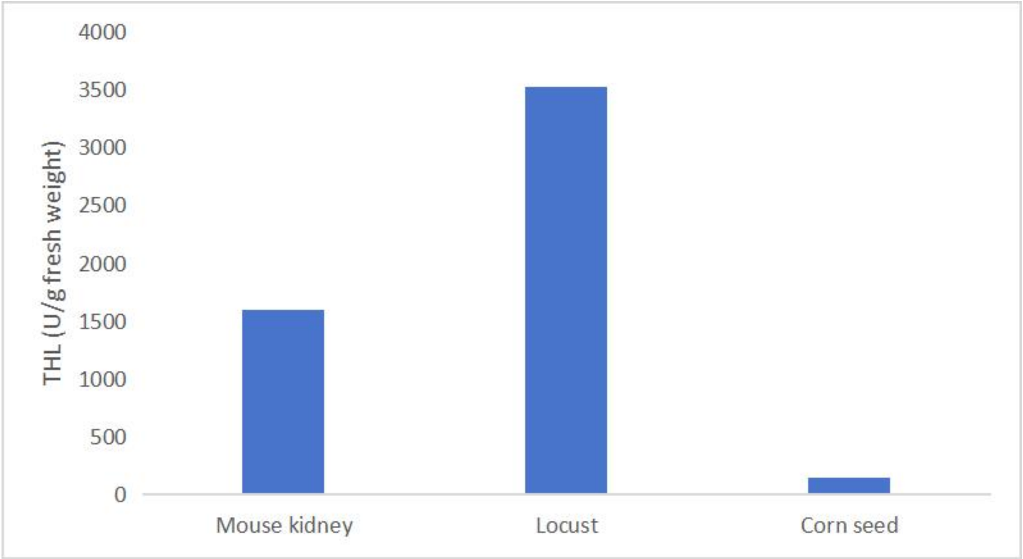

Figure 1. Determination of THL content in mouse kidney, locust and corn seed by this kit.

Recommended Products

Catalog No.	Product Name
KTB1410	CheKine™ Micro Alanine Aminotransferase (ALT/GPT) Activity Assay Kit
KTB1420	CheKine™ Micro Aspartate Aminotransferase (AST/GOT) Activity Assay Kit
KTB1430	CheKine™ Micro Proline (PRO) Content Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.

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