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Trehalase(THL) Activity Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Microplate Reader/ Spectrophotometer

Cat No: AK0217

Size:100T/48S

Components:

Extract solution: Liquid 100 mL×1. Storage at 4℃ .

Reagent I: Liquid 10 mL×1. Storage at 4℃ .

Reagent II: Powder×1. Storage at 4℃ . Dissolve with 2 mL of Reagent I before use. Prepare when the solution will be used.

Reagent III: Liquid 13 mL×1. Storage at 4℃ .

Reagent IV: Liquid 13 mL×1. Storage at room temperature.

Standard: Powder×1. Contain 10 mg of anhydrous glucose (dry weight loss < 0.2%). Dissolve the standard with 1 mL of distilled water to generate a 10 mg/mL glucose solution standard, store at 4 ℃ and use within one week. Or it can be dissolved in saturated benzoic acid solution for a longer time.

Standard solution: Dilute the 10 mg/mL glucose solution standard to 1, 0.8, 0.6, 0.4, 0.2 mg/mL with distilled water.

Product Description:

Trehalase(THL, EC 3.2. 1.28) is an enzyme found broadly in animals, plants, microorganisms and cultured cells. The main function of THL is to break down trehalose to produce glucose and directly use it for the energy supply of organism.

THL converts soluble trehalose to reducing sugar. 3.5-Dinitrosalicylic acid is reduced to brown-red amino compound by co-heating with reducing sugar. The brown-red amino compound has a maximum absorption peak at 540 nm and the absorbance ratio is in direct proportion to the contents of reducing sugar. In this kit, the THL activity is quantified by measuring the color development at 540 nm.

Reagents and Equipments Required but Not Provided:

Spectrophotometer/ microplate reader, water bath, desk centrifuge, adjustable transferpettor, micro glass cuvette/96 well flat-bottom plate, mortar/homogenizer, ice and distilled water.

Procedure:

1. Bacteria or cells

Collecting bacteria or cells into the centrifuge tube, after centrifugation discard supernatant. Suggest add 1 mL of Extract solution to 5 million of bacteria or cells. Use ultrasonication to splitting bacteria and cells (place on ice, ultrasonic power 20%, working time 3s, interval 10s, repeat for 30 times). Centrifuge at 8000 g for 10 minutes at 4℃ to remove insoluble materials and take the supernatant on ice before testing.

2. Tissue

Add 1 mL of Extract solution to 0.1 g of tissue, and fully homogenized on ice bath. Centrifuge at 8000 g for 10 minutes at 4℃ to remove insoluble materials, and take the supernatant on ice before testing.

3. Serum (plasma)

Add 0.9 mL of Extract solution to 0.1 mL of liquid sample, and fully homogenized on ice bath. Centrifuge at 8000 g for 10 minutes at 4℃ to remove insoluble materials, and take the supernatant on ice before testing.

II. Detection

1) Preheat microplate reader or spectrophotometer for 30 minutes, adjust the wavelength to 540 nm, set zero with distilled water.

2) Add the following reagents in 1.5 mL EP tubes:

Reagent	Contrast tube (C)	Test tube (T)	Standard tube (S)	Blank tube (B)
Sample (μL)	40	40	-	-
Standard solution (μL)	-	-	40	-
Distilled water (μL)	-	-	-	40
Reagent I (μL)	70	50	70	70
Reagent II (μL)	-	20	-	-
Mix thoroughly and incubate accurately at 37℃(mammal) or 25℃(other species) water bath for 10minutes.				
Reagent III (μL)	95	95	95	95
Reagent IV (μL)	95	95	95	95
Mix thoroughly and place the tubes in a boiling water (100℃) bath for 5 minutes (cover tightly to prevent moisture loss) and cooling rapidly. Take 200 μL of reaction solution into micro glass cuvette or 96 well flat-bottom plate to detect the absorbance at 540 nm, record as A_C, A_T, A_S and A_B respectively. $\Delta A_T=(A_T- A_C)$, $\Delta A_S=(A_S-A_B)$. Standard curve: The concentration of standard solution as y-axis, ΔA_S as x-axis, obtain the equation y=kx+b. Take ΔA_T to the equation to acquire y (mg/mL) value.				

III. Calculation:

1) Tissue protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1 μg of glucose in the reaction system per minute every mg protein.

THL Activity(U/mg prot)=1000× y÷T÷Cpr =100× y ÷Cpr

2) Tissue weight

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1 μg of glucose in the reaction system per minute every g tissue.

THL Activity(U/g weight)=1000×y÷T÷(W÷Ve)=100 ×y÷W

3) Bacteria or cultured cells

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1 μg of glucose in the reaction system per minute every 10^4 bacteria or cultured cells.

THL Activity(U/10^4 cell)=1000×y÷T÷(500÷Ve)=0.2×y

4) Serum (plasma)

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1 μg of glucose in the reaction system per minute every mL serum(plasma).

THL Activity(U/mL)=1000×y÷T÷(Vs÷Ve)=1000×y

1000: 1 mg/mL=1000 μg/mL

T: Reaction time (min), 10 minutes;

Vs: Sample volume (mL), 0.1 mL;

Ve: Extract solution volume, 1 mL;

Cpr: Supernatant sample protein concentration (mg/mL);

W: Sample weight, g;

500: 5 million cells or bacteria.

Recent Product Citations：

[1] Yan P, Wen C, Zhang S, et al. A toxicological, metabonomic and transcriptional analysis to investigate the property of mulberry 1-deoxynojirimycin against the growth of Samia cynthia ricini[J]. Pesticide biochemistry and physiology, 2018, 152: 45-54.

[2] Jing Li,Yabing Duan,Chuanhong Bian,et al. Effects of validamycin in controlling Fusarium head blight caused by Fusarium graminearum: Inhibition of DON biosynthesis and induction of host resistance. Pesticide Biochemistry and Physiology. January 2019;153:152- 160.(IF2.87)

[3] Goddijn O J M, Verwoerd T C, Voogd E, et al. Inhibition of trehalase activity enhances trehalose accumulation in transgenic plants[J]. Plant physiology, 1997, 113(1): 181- 190.

[4] Küenzi M T, Fiechter A. Changes in carbohydrate composition and trehalase-activity during the budding cycle of Saccharomyces cerevisiae[J]. Archiv für Mikrobiologie, 1969, 64(4): 396-407.

References：

[1] Goddijn O J M, Verwoerd T C, Voogd E, et al. Inhibition of trehalase activity enhances trehalose accumulation in transgenic plants[J]. Plant physiology, 1997, 113(1): 181- 190.

[2] Küenzi M T, Fiechter A. Changes in carbohydrate composition and trehalase-activity during the budding cycle of Saccharomyces cerevisiae[J]. Archiv für Mikrobiologie, 1969, 64(4): 396-407.

Related Products：

AK0291/AK0290	Glucogen Content Assay Kit
AK0211/AK0210	Cellulase(CL) Activity Assay Kit
AK0295/AK0294	Trehalose Content Assay Kit
AK0223/AK0222	Blood Glucose Content Assay Kit
AK0213/AK0212	Sorbitol Dehydrogenase(SDH) Activity Assay Kit