Trehalase(THL) Activity Assay Kit

Note: The reagents of this product are subject to change.Please note and strictly follow this instruction.

Operation Equipment: Microplate Reader/Spectrophotometer

Cat No: BC2515

Size:100T/48S

Components:

Extract solution: Liquid 100 mL×1. Storage at 2-8℃.

Reagent I: Liquid 10 mL×1. Storage at 2-8℃.

Reagent II: Powder×2. Storage at 2-8℃. Take 1 tube and add 0.6 ml reagent I before use. Prepare when the solution will be used. The unused reagent can be stored at 4℃ for 1 week.

Reagent III: Liquid 13 mL×1. Storage at 2-8℃.

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

Standard: Powder×1. Storage at 2-8℃.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 2 weeks. Or it can be dissolved in saturated benzoic acid solution for 4 weeks.

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.

The 3,5-dinitrosalicylic acid method was used to determine the amount of reducing sugars produced by THL-catalyzed seaweed sugars. The amount of reducing sugar and the depth of color of the reaction solution are proportional to the amount of reducing sugar within a certain range, thus determining the level of THL activity.

Reagents and Equipments Required but Not Provided:

Spectrophotometer/microplate reader, water bath/constant temperature incubator, 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 200w, 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. The spectrophotometer needs to be zeroed with distilled water.

2) Dilute the 10mg/mL standard solution with distilled water to 1, 0.8, 0.6, 0.4, 0.2mg/mL

3) 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. $\Delta A_T=(A_T-A_C)$, $\Delta A_S=(A_S-A_B)$. Each Test tube shall be provided with a control tube. The standard tube and blank tube only need to be measured 1-2 times.				

III. Calculation:

1) Standard curve

A standard curve was created from the concentration of the standard tube (y, mg/mL) and the absorbance ΔAs (x, ΔAs). Based on the standard curve, ΔA (x, ΔAt) was brought into the equation to calculate the sample concentration (y, mg/mL).

2) 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 milligram protein.

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

3) 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 gram tissue.

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

4) 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

5) 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 milliliter 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:

BC0340/BC0345 Glucogen Content Assay Kit

BC2540/BC2545 Cellulase(CL) Activity Assay Kit

BC0330/BC0335	Trehalose Content Assay Kit
BC2490/BC2495	Blood Glucose Content Assay Kit
BC2530/BC2535	Sorbitol Dehydrogenase(SDH) Activity Assay Kit

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