

## Trehalase(THL) Activity Assay Kit

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

**Operation Equipment:** Spectrophotometer

**Cat No:** BC2510

**Size:** 50T/24S

### Components:

**Extract solution:** Liquid 50 mL×1. Storage at 2-8°C.

**Reagent I:** Liquid 30 mL×1. Storage at 2-8°C.

**Reagent II:** Powder×2. Storage at 2-8°C. Take 1 tube and add 1.2 ml reagent I before use.

Prepare when the solution will be used. The unused reagent can be stored at 4°C for 1 week.

**Reagent III:** Liquid 30 mL×1. Storage at 2-8°C.

**Reagent IV:** Liquid 30 mL×1. Storage at room temperature.

**Standard:** Powder×1. Storage at 2-8°C. 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 °C 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, water bath/constant temperature incubator, desk centrifuge, adjustable transferpettor, 1 mL glass cuvette, 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 (placed on ice, ultrasonic power 200w, working time 3s, interval 10s, repeat for 30 times). Centrifuge at 8000×g for 10 minutes at 4°C 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°C 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°C to remove insoluble materials, and take the supernatant on ice before testing.

**II. Detection**

- 1) Preheat spectrophotometer for 30 minutes, adjust the wavelength to 540 nm, set zero 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)	180	180	-	-
Standard solution (μL)	-	-	180	-
Distilled water (μL)	-	-	-	180
Reagent I (μL)	300	220	300	300
Reagent II (μL)	-	80	-	-
Mix thoroughly and incubate accurately at 37°C(mammal) or 25°C(other species) water bath for 10minutes.				
Reagent III (μL)	400	400	400	400
Reagent IV (μL)	400	400	400	400
Mix thoroughly and place the tubes in a boiling water (100°C) bath for 5 minutes (cover tightly to prevent moisture loss) and rapid cooling by ice bath. Detect the absorbance at 540 nm, record as A <sub>C</sub> , A <sub>T</sub> , A <sub>S</sub> and A <sub>B</sub> 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  $\Delta A_S$  (x,  $\Delta A_S$ ). Based on the standard curve,  $\Delta A$  (x,  $\Delta A_T$ ) 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.

$$\text{THL Activity(U/mg prot)}=1000 \times y \div T \div C_{pr} = 100 \times y \div C_{pr}$$

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.

$$\text{THL Activity(U/g weight)}=1000 \times y \div T \div (W \div V_e) = 100 \times y \div W$$

#### 4) Bacteria or cultured cells

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

$$\text{THL Activity}(U/10^4 \text{ cell})=1000 \times y \div T \div (500 \div V_e)=0.2 \times y$$

#### 5) Serum (plasma)

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1  $\mu\text{g}$  of glucose in the reaction system per minute every milliliter serum(plasma).

$$\text{THL Activity}(U/\text{mL})=1000 \times y \div T \div (V_s \div V_e)=1000 \times y$$

$$1000: 1 \text{ mg/mL}=1000 \mu\text{g/mL}$$

T: Reaction time (min), 10 minutes;

$V_e$ : Extract solution volume, 1 mL;

$V_s$ : Sample volume (mL), 0.1 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] Kuenzi 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] Kuenzi 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