Trehalose Synthase (TS) Activity Assay Kit

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

Operation Equipment: Spectrophotometer/Microplate reader

Catalog Number: BC4955

Size: 100T/48S

Components:

Reagent	Size	Storage
Extract solution	Solution 60 mL×1	4℃
Reagent I	Solution 6mL×1	4℃
Reagent II	Powder×2	4℃
Reagent III	Solution 15 mL×1	4℃
Reagent IV	Solution 15 mL×1	4℃
Standard	Powder×1	4℃

Solution preparation:

Reagent I: Add 6 mL of distilled water before use, mix thoroughly. The unused reagents should be stored at 4℃ in 2weeks.

Working solution: Mix Reagent III and Reagent IV according to 1:1 before use. According to the actual amount of sample, prepare when the solution will be used.

Standard solution: Add 1 mL distilled water to prepare the 50 μmol/mL maltose standard solution before use. The unused reagents should be stored at 4℃ in 2weeks. Then dilute 8 times with distilled water to 6.25 μmol/mL maltose standard solution (it is recommended to absorb 25 μL of 50 μmol/mL maltose standard solution, add 175 μL distilled water and mix thoroughly) for test.

Product Description：

Trehalose is a functional oligosaccharide. It has the characteristics of non-reduction, moisture retention, heat the acid stability, anti-freezing and so on. It is one of the important anti stress substances produced by cells in adverse environmental conditions. It has nonspecific protective effect on biological macromolecules and tissues.

Trehalose synthase (TS) can catalyze maltose to trehalose. It is one of the key pathways of trehalose biosynthesis. In this kit, the remaining maltose is decomposed into glucose by glucoamylase. Use the glucose oxidase method to determine the glucose content. The activity of trehalose synthetase can be shown by the reduced amount of maltose.

Reagents and Equipment Required but Not Provided：

Spectrophotometer/microplate reader, desk centrifuge, transferpettor, mico glass cuvette/96 well flat -bottom plate, mortar/homogeniser, ice and distilled water.

Procedure

I. Sample preparation:

A. Bacteria or cells

Collecting bacteria or cells into the centrifuge tube, the supernatant is discarded after centrifugation. It is suggested to take about 5 million bacteria/cell and add 1 mL of Extract solution. Bacteria or cell is splitted by ultrasonication (Power: 200 W, work time 3s, interval 9s, repeat for 30 times). Centrifuge at 8000 g for 10 minutes at 4℃.Take the supernatant for test. (If the supernatant is not clear enough, it is recommended to repeat the above centrifugation steps.)

B. Tissue

It is suggested to take about 0.1 g of tissue and add 1 mL of Extract solution, fully grinding on ice. Centrifuge at 8000 g for 10 minutes at 4℃. Take the supernatant for test. (If the supernatant is not clear enough, it is recommended to repeat the above centrifugation steps.)

II. Determination procedure：

1. Preheat spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 505 nm, set zero with distilled water.
2. Add reagents with the following list:

(1) Enzymatic reaction

Reagent（μL）	Contrast tube	Test tube	Standard tube	Blank tube
Sample	50	50	-	-
Reagent I	-	50	-	-
Standard	-	-	50	-
Distilled water	-	-	50	100
Mix thoroughly. React at 35℃ water bath for 2 h. Put it in boiling water bath for 5 min to terminate the reaction. Cool it to room temperature.			-	-
Reagent I	50	-	-	-
Reagent II	100	100	100	100
Mix thoroughly. React at 40℃ for overnight (more than 12 hours). Put it in boiling water bath for 5 min to terminate the reaction. Cool it to room temperature. Centrifuge at 10000 g for 10 minutes at 25℃. Take the supernatant for test.				

(2) Color reaction

Reagent（μL）	Contrast tube	Test tube	Standard tube	Blank tube
Supernatant	50	50	50	50
Working solution	180	180	180	180
Mix thoroughly. React at 37℃ for 30 min. Measure the absorbance at 505 nm in a 1 mL glass cuvette. Record as A_C、A_T、A_S与A_B，$\Delta A_T=A_C-A_T$、$\Delta A_S=A_S-A_B$。				

Note: Blank tube only need to test 1~2 times.

III. Calculations：

A. Protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes 1 nmol of maltose to produce 1 nmol of trehalose in the reaction system per minute every milligram protein.

TS (U/mg prot)＝C_S×ΔA_T÷ΔA_S×V_S÷ (V_S×Cpr) ÷T×F×1000÷2= 26.041×ΔA_T÷ΔA_S÷Cpr×F

B. Sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes 1 nmol of maltose to produce 1 nmol of trehalose in the reaction system per minute every gram tissue.

TS (U/g weight)＝C_S×ΔA_T÷ΔA_S×V_S÷ (V_S÷V_E×W)÷T×F×1000÷2= 26.041×ΔA_T÷ΔA_S÷W×F

C. Cell amount

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes 1 nmol of maltose to produce 1 nmol of trehalose in the reaction system per minute every 10^4 bacteria or cells.

TS (U/10^4 cell)＝C_S×ΔA_T÷ΔA_S×V_S÷ (V_S÷V_E×cells)÷T×F×1000÷2= 26.041×ΔA_T÷ΔA_S÷cells×F

C_S: Concentration of Standard, 6.25 μmol/mL=6.25×10^3 nmol/mL;

Vs: Sample volume, 0.05 mL;

V_E: Extract solution volume, 1 mL;

T: Reaction time, 120 minutes;

Cpr: Sample protein concentration, mg/mL;

W: Sample weight, g;

F: Dilution times;

1000: Unit conversion factor: 1 μmol=1000 nmol;

Cells: Total number of bacteria or cells, 10^4.

Note:

When the absorbance value is greater than 1.0, it is recommended to dilute the sample and measure it. Note that multiply the dilution factor in the calculation formula.

Experimental example

1. Weigh 0.1 g mouse liver. Add 1 mL of Extract solution to homogenize. Take the supernatant for test. Dilute the supernatant two times with distilled water. Follow the measurement procedure. Measure with 96 well flat-bottom plate. Calculate ΔA_T=A_C-A_T=1.177-0.536=0.641. Substitute the ΔA_T into the standard curve, and calculate the deenzyme activity according to the sample quality:

   TS (U/g weight) = 26.041×ΔA_T÷ΔA_S÷W×F =339.965 U/g weight.

2. Weigh 0.1 g kelp. Add 1 mL of Extract solution to homogenize. Take the supernatant for test. Follow the measurement procedure. Measure with 96 well flat-bottom plate. Calculate ΔA_T=A_C-A_T=1.366-0.793=0.573. Substitute the ΔA_T into the standard curve, and calculate the deenzyme activity according to the sample quality:

TS (U/g weight) = $26.041 \times \Delta A_T \div \Delta A_S \div W \times F$ =151.950 U/g weight.

Related products

BC0330/BC0335 Trehalose Content Assay Kit

BC2510/BC2515 Trehalase(THL) Activity Assay Kit

Tel: 86-010-50973105 https://www.solarbio.net E-mail: info@solarbio.com