Sucrose Phosphorylase (SP) Activity Assay Kit

Note: Before the experiment, it is recommended to select 2-3 sample with large expected differences for pre-experiment.

Operation Equipment: Spectrophotometer

Catalog Number: BC0450

Size: 50T/48S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation Condition
Extract solution	Liquid 60 mL×1	2-8℃
Reagent I	Liquid 20 mL×1	2-8℃
Reagent II	Powder ×1	2-8℃
Reagent III	Liquid 2 mL×1	2-8℃
Reagent IV	Powder ×2	-20℃
ReagentV	Powder ×1	-20℃
ReagentVI	Powder ×3	-20℃
ReagentVII	Powder ×4	-20℃

Solution Preparation:

1. **Reagent II:** Dissolve it with 15 mL of distilled water before use. It can be stored for 4 weeks at 2-8℃.
2. **Reagent IV:** Dissolve one with 1.5 mL of distilled water before use. It can be sub-packaged and stored at -20℃ for 2 weeks. Avoid repeated freezing and thawing during storage.
3. **Reagent V:** Place the powder in a glass tube inside the bottle. Dissolve it with 10 mL of distilled water before use. It can be sub-packaged and stored at -20℃ for 4 weeks. Avoid repeated freezing and thawing during storage.
4. **Reagent VI:** Dissolve one with 1 mL of distilled water before use. It can be sub-packaged and stored at
   -20℃ for 2 weeks. Avoid repeated freezing and thawing during storage. Before use, dilute the reagent VI according to the ratio of reagent VI: distilled water=1:1, ready for use.
5. **Reagent VII:** Dissolve one with 0.7 mL distilled water before use. It can be sub-package and store for 2 weeks at -20℃. Before use, dilute the reagent VII according to the ratio of reagent VII: distilled water=1:1, ready for use.

Note: Reagents VI and VII are freeze-dried reagents, and there may be significant or even small differences in the amount of reagents observed by the naked eye between different bottles. This phenomenon does not affect the use, and the actual quality is the same.

Product Description:

Sucrose phosphorylase (SP) (EC2.4.1.7) is mainly found in microorganisms and plants, belonging to

the glycosyl hydrolase 13 family. It is an enzyme that catalyzes the transfer of glucosidic bonds and can catalyze the synthesis of 1-phosphate glucose from sucrose and inorganic phosphate. This enzyme mainly uses sucrose and 1-phosphate glucose as donors, and various substances such as polyhydroxy sugars and sugar alcohols, phenolic hydroxyl groups, carboxyl groups, etc. as acceptors to catalyze the synthesis of various glycosides.

SP can catalyze sucrose to produce 1-phosphate glucose, which is then converted to 6-phosphate glucose by glucose phosphate mutase. Under the action of 6-phosphate glucose dehydrogenase, $NADP^+$ is reduced to form NADPH, resulting in an increase in 340nm light absorption value. The increase rate of 340nm absorbance is used to reflect SP activity.

Reagents and Equipment Required but Not Provided：

Ultraviolet spectrophotometer, low temperature centrifuge, constant temperature incubator/water bath, adjustable pipette, mortar/homogenizer, 1 mL quartz cuvette, ice and distilled water.

Procedure

I. Sample preparation:

1. Tissue sample: according to the proportion of tissue weight (g): extraction solution volume (mL) of 1:5-10 to extract. It is suggested that 0.1 g of tissue with 1 mL of Extract solution and fully homogenized on ice bath. Centrifuge at 10000 ×g for 10 minutes at 4℃ to remove insoluble materials, and take the supernatant on ice before testing.
2. Bacteria or cells: collecting bacteria or cells into EP tube, centrifuge at 3000rpm for 5 minutes and discard the supernatant. According to the ratio of the number of cells or bacteria (10^4) to the volume of extraction solution (mL) of 500-1000:1 (it is recommended to add 1 mL of Extract solution to 5 million cells or bacteria). Use ultrasonication to splitting bacteria and cells (placed on ice, ultrasonic power 200W, working time 3 s, interval 7 s, for 3 min). Centrifuge at 10000 ×g for 10 minutes at 4℃, and take the supernatant on ice for testing.
3. Liquid sample: detect sample directly. If the liquid is turbid, centrifuge and take the supernatant for measurement.

II. Determination procedure：

1. Preheat ultraviolet spectrophotometer for 30 minutes, adjust wavelength to 340 nm, set zero with distilled water.
2. Preheat Reagent I at 37℃ for 10 minutes.
3. Add reagents with the following list:

Reagent (μL)	Test tube (A_T)	Blank tube (A_B)
Reagent I	325	425
Reagent II	250	250
Reagent III	25	25
Reagent IV	50	50

Reagent V	50	50
Reagent VI	100	100
Reagent VII	100	100
Mix thoroughly，37℃ water bath preheating 5min		
sample	100	-

Add the above reagents to the cuvette and quickly mix by pipetting. Record the absorbance value A_T1 (A_B1) of the tube in 15s, quickly place it in 37℃ water bath or incubator for 2 minutes, take it out and quickly dry it and measure the absorbance value A_T2（A_B2）in 2min15s ,Calculate $\Delta A = (A_T2-A_T1)-(A_B2-A_B1)$.

Note: Blank tube only need to test 1-2 times. If the number of samples is too large, you can also mix Reagent I to Reagent VII according to the above ratio and then perform the measurement.

III. Calculations:

A. Protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme produce 1 nmol NADPH per minute every milligram protein in 37℃.

SP (U/mg prot)＝$=\Delta A \div \varepsilon \div d \times V_R \times 10^9 \div (V_S \times Cpr) \div T = 803.85 \times \Delta A \div Cpr$

B. Sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme produce 1 nmol NADPH per minute every gram tissue in 37℃.

SP (U/g weight) $=\Delta A \div \varepsilon \div d \times V_R \times 10^9 \div (V_S \div V_E \times W) \div T = 803.85 \times \Delta A \div W$

C. Cell amount

Unit definition: One unit of enzyme activity is defined as the amount of enzyme produce 1 nmol NADPH per minute every 10^4 bacteria or cells in 37℃.

SP (U/10^4 cell)$=\Delta A \div \varepsilon \div d \times V_R \times 10^9 \div (V_S \div V_E \times N) \div T = 803.85 \times \Delta A \div N$

ε : NADPH molar extinction coefficient, 6220 L/mol/cm;

D: Cuvette light path, 1 cm;

V_R: Total reaction volume, 0.001 L;

V_S: Add sample volume, 0.1 mL;

V_E: Extract solution volume, 1 mL;

W: Sample weight, g;

Cpr: Protein concentration of sample, mg/mL;

T: Reaction time, 2min;

10^9: 1mol=10^9nmol;

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N: cell number, 10^4.

Note:

If the measured absorbance value A>1.5, it is recommended to dilute the sample before measuring, and multiply the dilution factor in the calculation formula. If the measured absorbance value is low or close to the blank OD value, it is recommended to increase the sample volume before performing the measurement.

Experimental example:

1. Take 0.1 g of potatoes, add 1 mL of Extract solution, homogenize in ice bath, centrifuge at 10000 ×g for 10 minutes at 4℃, take the supernatant and place on ice for testing. Use 1mL quartz cuvette to operate according to the determination steps, calculate ΔA= (0.5620-0.4300) – (0.059-0.059) =0.132, according to the formula calculated activity:

   SP activity (U/g weight) =803.85×ΔA÷W=1061.1 U/ g weight

2. Take 0.1 g of black rice, add 1 mL of Extract solution, homogenize in ice bath, 10000 ×g, Centrifuge at 10000 ×g for 10 minutes at 4℃, take the supernatant and place on ice for testing. Use 1mL quartz cuvette to operate according to the determination steps, calculate ΔA= (0.7290-0.6030) – (0.059-0.059) = 0.126, calculate the activity according to the formula:

   SP activity (U/g weight) = 803.85×ΔA÷W=1012.85 U/ g weight

Reference:

[1] Balasubramaniam K, Kannangara P N. Sucrose phosphorylase and invertase activities in bacteria [J]. Journal of the National Science Foundation of Sri Lanka, 1982, 10(2): 169-180.

[2] Broek L V, Boxtel E L, Kievit R P. et al. Physico-chemical and transglucosylation properties of recombinant sucrose phosphorylase from Bifidobacterium adolescentis DSM20083 [J]. Applied Microbiology and Biotechnology, 2004, 65: 219-227.

[3] Malik A, Ishikawa S, Sahlan M. et al. Screening for sucrose phosphorylase in exopolysaccharide producing-lactic acid bacteria reveals spasewrs-3(1) in Leuconostoc mesenteroides isolated from sugar containing-beverage ‘wedang ronde’ from Indonesia [J]. African Journal of Biotechnology, 2011, 10(74): 16915-16923.

Related products:

BC0580/BC0585 Sucrose Synthetase (SS) Activity Assay Kit
BC0570/BC0575 Neutral Invertase (NI) Activity Assay Kit
BC0560/BC0565 Acid Invertase (AI) Activity Assay Kit
BC0600/BC0605 Sucrose Phosphoric Acid Synthetase (SPS) Activity Assay Kit